

The Synthesis of Imidazole Fatty Acid Conjugates as Inhibitors of Apoptosis

By

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Abstract

Ionizing radiation is known to initiate apoptosis in mammalian cells by causing the transformation of cytochrome c into a peroxidase, which results in the specific peroxidation of the mitochondrial phospholipid cardiolipin. Here we report the design and synthesis of 8 imidazole fatty acid derivatives that bind to the cyt c:CL complex and inhibit the peroxidase activity required for the initiation of apoptosis. We postulate that imidazole acts as a sixth ligand to the haem iron and stops the interaction with H₂O₂. Two mitochondrially directed analogues (3-hydroxypropyl)triphenylphosphonium esters) of 12-imidazole-stearic acid and 12-imidazole-oleic acid not only were demonstrated to be peroxidase inhibitors in vitro, but were also extraordinarily effective in protecting mice from lethal doses (9 Gy) of ionization radiation. We studied the structure activity relationship to a group of triphenyl phosphonium derivatives containing imidazole at different positions on the fatty acid chain, and observed that the C₈-imidazole stearate analogue had marginally better activity than the others. But overall, the structure activity result were remarkable “flat” with all compounds prepared having rather similar inhibitory strength. We also synthesized carnitine mono and di-esters of 12-imidazole fatty acids but full biological data is not yet available for these compounds.

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List of Abbreviations

Ac	Acetyl
DCC	N,N'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DCU	N,N'-Dicyclohexylurea
DMAP	4-(Dimethylamino)pyridine
DMF	N,N-Dimethylformamide
EtOAc	Ethyl acetate
EtOH	Ethanol
FAB	Fast Atom Bombardment
HRMS	High Resolution Mass Spectrometry
MeOH	Methanol
MS	Mass Spectroscopy
MsCl	Methanesulfonyl Chloride
NMR	Nuclear Magnetic Resonance
RT	Room Temperature
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TPP	Triphenylphosphine
TFA	Trifluoroacetic Acid

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1. Introduction

The March 11th, 2011 earthquake and tsunami in Japan caused extensive damage to the Fukushima Daiichi nuclear power generating station. This disaster brought to the public's attention the very serious concerns of human exposure to ionizing radiation. However, humans are exposed to radiation in other ways: airplane flight crews are exposed to cosmic radiation during high altitude flights, and patients receiving radiation therapy for solid tumours can be received very high doses of focused radiation on particular tissues. Ionizing radiation induces controlled, or programmed, cell death process called apoptosis.¹ This is thought to be initiated by the generation of reactive oxygen species (ROS).² Unfortunately, during the irradiation of tumour cells, normal cells are also affected, leading to the loss of non-diseased tissue. Cancer patients have been treated with electromagnetic radiation (photons), such as X-rays and gamma rays, and particulate radiation, usually electrons and, to a lesser extent, neutrons and protons.³ There are no effective solutions to protect non-cancerous cells from the damaging effects of ionizing radiation.

It has been shown that the concentrations of vitamins C and E in mice bone marrow were reduced following total body exposure to X-ray radiation.⁴ Radiation induced apoptosis in cancer radiotherapy was reduced by antioxidants, vitamins E and C, phytochemicals and micronutrients like selenium.⁵ Antioxidant phytochemicals, including flavonoids, polyphenols, carotenoids and organosulfur compounds, have been shown to have varying degrees of effectiveness as radio-protective substances.^{6,7}

There is a need to understand the biological mechanisms of radiation-induced apoptosis. Radiation affected cells undergo apoptosis by several mechanisms. Induction of apoptosis by

ionizing radiation can occur in different cellular compartments, including the nucleus, cytosolic elements (organelles) and the plasma membrane⁸ and the generation of reactive oxygen species (ROS) is thought to be a common initiating event.

1.1 Apoptosis

The word “apoptosis” is from the ancient Greek words *apo* - from/off/without, and *ptosis* – falling.⁹ The term “apoptosis” was first introduced by John Kerr in 1972.¹⁰ It refers to the formation in a cell, under various initiating causes, of a morphological feature called “apoptotic bodies”.^{11,12} The German scientist Carl Vogt described the principle of apoptosis in 1842¹³ and the anatomist Walther Flemming presented a more precise description of the process of programmed cell death in 1885.¹⁴ Sydney Brenner, H. Robert Horvitz and John E. Sulston jointly received the Nobel Prize for Medicine or Physiology in the year of 2002 for their discovery on gene regulation of organ development and programmed cell death or apoptosis in the nematode *Caenorhabditis elegans*.

Cell death by apoptosis is an active process. It plays a key role in the development of multicellular organisms. Cells that have been produced in excess during early growth and development can undergo programmed cell death to contribute to the forming of organs and tissues.¹³ Apoptosis regulates and maintains cell populations in tissues during both normal physiological and pathological conditions.¹⁵ In the human body around 100,000 cells are produced each second by the process of mitosis and the same number of cells die by the process of apoptosis.¹⁶ During the development of the brain, half of the neurons that are initially formed will die by the time the adult brain is formed.^{13, 17}

Damage to healthy cells can also cause apoptosis. For instance, if a cell's DNA is damaged to some extent, then it will try to repair it. If the cell is unable to repair the damaged DNA, then apoptosis may be initiated. Such a process is a protection against cancer since cells with damaged DNA can lose control of growth processes and may turn cancerous.

Scientists have shown great interest in apoptosis since a number of diseases are known to be caused or complicated by increased levels of apoptosis, including degenerative diseases such as Parkinson's, Alzheimer's, spinal muscular atrophy and AIDS. Decreased levels of apoptosis are known to be involved in cancer (DNA mutations in critical control proteins such as p53 and Bcl-2) or autoimmune diseases (type I diabetes or encephalomyelitis). Apoptosis can also be triggered by toxins and other cellular stresses such as oxidative stress or alcohol.¹¹

1.2 Morphological changes of apoptosis

Apoptotic cells can be identified by a distinct set of biochemical and physical changes.¹⁵ During early stages of apoptosis, the apoptotic cell shrinks, shows membrane deformations and loses contact from the neighbouring cells. Its chromatin condenses and marginates to the nuclear membrane. As the process continues, the plasma membrane shows distinct blebbing and budding. Finally, the cell is divided into compact membrane-enclosed structures called "apoptotic" bodies. These bodies have cytosol, the condensed chromatin and organelles. These are shown in the **Figure 1**. Macrophages engulf the apoptotic bodies and these apoptotic bodies are removed from the tissue without causing an inflammatory response.

Cells can also die via necrosis, usually after suffering a major physiological insult.¹⁸ This results in a loss of membrane integrity, swelling and breakup of the cells. The release of cellular contents into the surrounding environment can be observed during necrosis. This can damage the

surrounding cells and initiate a strong inflammatory response within the tissue.¹⁸ **Figure 1** shows the generalized pathways involved in the processes of apoptosis and necrosis.

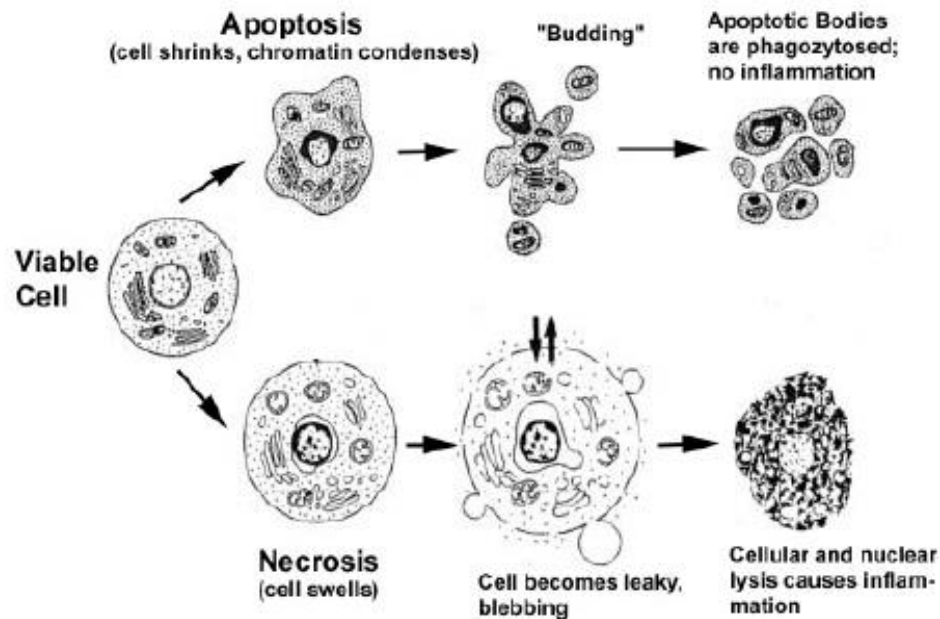


Figure 1: Steps involved in the process of apoptotic and necrotic cell death.¹⁵

Cellular shrinking, chromatin condensation and margination at the nuclear periphery are observed in apoptosis, followed by the eventual formation of membrane-bound apoptotic bodies that contain organelles, cytosol and nuclear fragments which are phagocytosed without triggering inflammatory processes. During necrosis, the cell swells, becomes leaky, and finally is disrupted and releases its contents into the surrounding tissue resulting in inflammation.^{15,19}

1.3 Mitochondria

The word mitochondrion is derived from the Greek words *mitos* (μίτος) meaning thread, and *chondrion* (χονδρίον) meaning granule. Most eukaryotic cells contain mitochondria which are absent from prokaryotic cells. A mitochondrion is an organelle with inner and outer membranes.²⁰ The size of these organelles range from 0.5 to 1.0 μm in diameter. Mitochondria

are cellular power plants within the cell, converting high chemical potential molecules such as fatty acids and sugars, into more usable chemical forms such as adenosine triphosphate (ATP).²¹ Mitochondria are also involved in a wide range of other processes like cell signalling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth.²² It is also now becoming increasingly clear that it is in the mitochondria where the first key steps occur that lead a cell down the apoptotic cascade.²³

1.4 Mitochondria and cell death

There are three general mechanisms by which mitochondria can participate in the initiation and promotion of apoptosis, and these effects might be interrelated with each other. These mechanisms are: (i) change of cellular reduction-oxidation (redox) potential, (ii) disruption of electron transport, oxidative phosphorylation, and adenosine triphosphate (ATP) synthesis and (iii) release of proteins that trigger activation of the caspase family of proteases.¹

1.5 Reactive oxygen species (ROS)

Reactive oxygen species (ROS) are short-lived diffusible species that include hydroxyl ($\text{HO}\bullet$), alkoxyl ($\text{RO}\bullet$) and peroxy ($\text{ROO}\bullet$) radicals. Some radicals, such as superoxide ($\text{O}_2^{\bullet-}$) and nitroxyl radical ($\text{NO}\bullet$) species, have long lifetimes of many seconds to minutes. The non-radicals hydrogen peroxide (H_2O_2), organic hydroperoxides (ROOH) and hypochlorous acid (HOCl) are also included in ROS. ROS can damage both DNA and proteins.²⁴ ROS may also act as chemical messengers. H_2O_2 is a secondary messenger²⁵ that is involved in receptor-mediated signalling pathways and transcriptional activation.²⁶ Hydrogen peroxide has been shown to induce apoptosis and this induction was prevented by catalase.²⁷

During the transfer of electrons to molecular oxygen during oxidative phosphorylation, an estimated 1 to 2% of electrons in the respiratory chain lose their way, most of them participating in formation of ROS.²⁸ The production of superoxide can be increased by anything that decreases the coupling efficiency of the electron transport chain.¹ Increased superoxide formation can lead to increased lipid peroxidation which is generally accepted to be an early chemical event in apoptosis.²⁹ Manganese superoxide dismutase converts superoxide anion $O_2^{\cdot-}$ into H_2O_2 and O_2 . In the presence of transition metals such as iron (Fe^{3+}), hydrogen peroxide can be converted to hydroxyl radical via Fenton chemistry.³⁰ This process has been shown in the **Figure 2**.

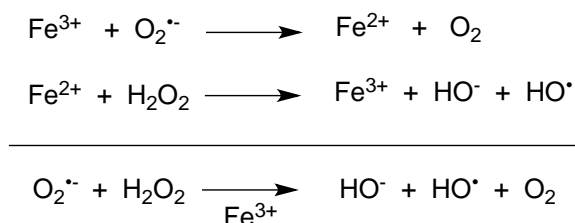


Figure 2: Redox processes in the presence of Fe^{3+} .

If a sequential process is assumed, initial released ROS from mitochondria can directly or indirectly (via ceramide generation) increase the gating potential of the pore (**Figure 3**) on mitochondria, rendering mitochondria both as a source and target of ROS.²⁴ The major sources of superoxide anion production in cells are mitochondria. Release of cyt c seems to be the significant event¹ in apoptosis initiation, as it is a required step for aggregation of the adapter molecule Apaf1. The pro-apoptotic member of the Bcl-2 family Bax protein is responsible for

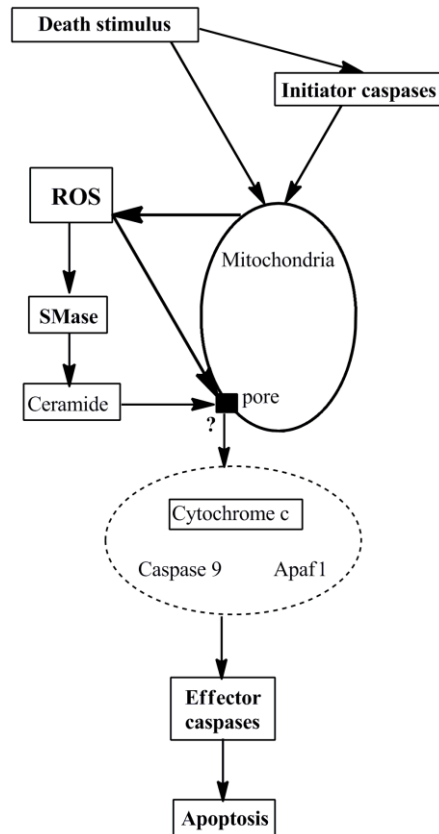


Figure 3: Role of ROS in apoptosis induction and source and target of mitochondria.²⁴

releasing cytochrome c from mitochondria.³¹ Cytochrome c exits from the mitochondrial membrane by a pore created by some Bcl-2 family members, including Bax.¹ The disruption of the mitochondrial potential by ROS and opening of the mitochondrial permeability pore¹¹ leads to the release of cytochrome c.³²

1.6 Cytochrome c (cyt c)

Cytochrome c is a small heme protein having 12 kDa molecular weight. It can be found in the inner membrane of the mitochondrion and is loosely associated with it.³³ Cytochrome c is a highly soluble protein, about 100 g/L at pH 7.0. The cyt c contains 104 amino acids and a heme group that is covalently bonded to the protein.³⁴ The cyt c is a chief component of the electron transport chain. It can undergo one-electron oxidation and reduction, but it does not bind to oxygen. It transfers electrons between Complexes III (Coenzyme Q - Cyt C reductase) and IV (Cyt C oxidase).³⁵ The structure of human cytochrome c protein is shown in **Figure 4**. The orange colored iron atom is coordinated with six ligands, the nitrogen atoms from the porphyrin ring contribute four ligands, and the sulphur atom of methionine-80 and nitrogen atom of histidine-18 residues form the fifth and sixth ligands.

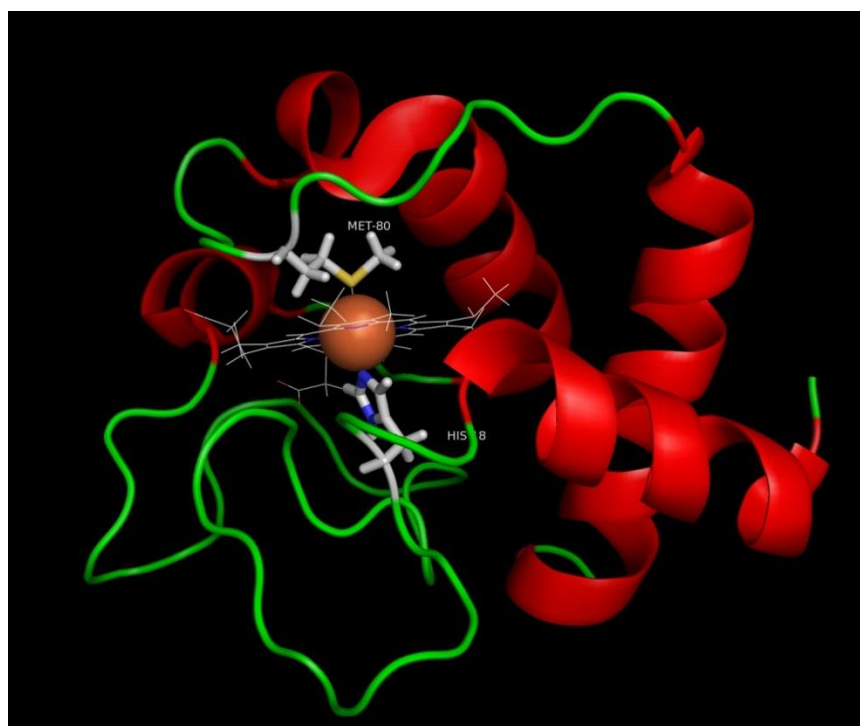


Figure 4: Human Cytochrome c protein.

1.7 Cardiolipin (CL)

Cardiolipin (IUPAC name: 1,3-bis(*sn*-3'-phosphatidyl)-*sn*-glycerol), is an important component of the mitochondrial inner membrane, where it comprises about 20% of the total lipid composition. CL was first isolated from beef heart in the early 1940s.³⁶ Both in mammalian and plant cells, CL is found in the inner mitochondrial membrane.³⁷ It is an essential component for the optimal function of many enzymes which are involved in mitochondrial energy metabolism. The generalized structure of cardiolipin is shown in **Figure 5**.

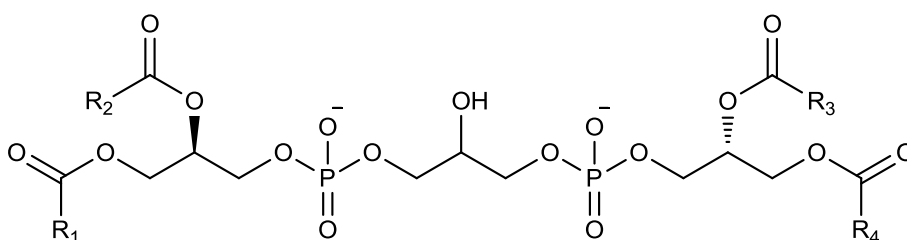


Figure 5: Structure of cardiolipin, where R₁, 2, 3 and 4 are 18-carbon chain fatty acid.

CL is a diphosphatidylglycerol lipid, in which there are three glycerol moieties; two phosphatidylglycerols connect with a glycerol backbone to form a dimer. It thus contains four acyl groups and two negative charges. Most CL in animal tissues contains 18-carbon fatty acyl chains with two unsaturated bonds, called linoleic acid.³⁸ The occurrence of these unsaturated fatty acids in mitochondria renders it the primary target of attack by free radicals.³⁹

CL is found primarily in membranes that also has the F₀F₁-ATPase. CL is the only eukaryotic phospholipid which is synthesized in mitochondria, where it remains until the cell dies. All other phospholipids are synthesized in the endoplasmic reticulum. CL has high binding affinity for the membrane proteins that are involved in the synthesis of ATP in the mitochondrion.⁴⁰

CL is in the inner membrane of mitochondria and is found in only small amounts in the rest of the cell. CL exists in contact with sites between the outer and inner membranes. The tBid protein targets this CL and leads to an increase in negative intrinsic curvature of the membrane, destabilizing the bilayer and likely facilitating cyt c leakage.⁴¹ This step is followed by the binding of CL to Bax/BaK, eliciting oligomerization and cristae remodeling that starts the destruction of the organelle.⁴⁰

CL can interact with the electron transport chain complexes that are involved in oxidative phosphorylation. It is important for optimal activity of the respiratory chain complexes.^{42, 43} CL peroxidation is an early event in the release of cytochrome c and caspase activation.⁴⁴ CL can play an important role for the integrity and function of the mitochondrial inner membrane and also associated with members of the apoptotic machinery including cytochrome c, Bid and caspase 8.⁴⁵

1.8 Aim & objectives

Cardiolipin (CL) peroxidation is the chief event leading to formation and release of pro-apoptotic bodies.⁴⁶ The catalyst for the peroxidation of the CL is cytochrome c (cyt c).⁴⁷ In normally functioning mitochondria, CL and cyt c are well separated, but in the initial stage of apoptosis, CL and cyt c form a cyt c/CL complex whose redox potential is -400 mV more negative than that of intact cyt c.⁴⁸ The cyt c/CL complex is formed by the trans-membrane redistribution of CL allowing a physical interaction of CL and cyt c.⁴⁷ The entire process is driven by few mitochondrial proteins. The cyt c can have two important interactions with phospholipids, one is an electrostatic interaction and other one is a hydrophobic interaction. These two kinds of interactions are necessary for cyt c to exhibit peroxidase activity. The initiation of apoptosis in irradiated cells is marked by a specific increase in the peroxidation of CL. As cyt c/CL complex formation initiates the apoptotic events, we are interested in targeting the same complex to stop the activity of the cyt c peroxidase and thus radiation-induced apoptosis. The cyt c has a heme-iron that is the catalytic site for peroxidase activity. Upon interacting with CL, cyt c partially unfolds and the heme-iron loses a methionine ligand.⁴⁹ Unfolding of the protein opens a crevice, allowing small molecules such as H₂O₂ access to the heme iron atom. We postulated that locking the heme-iron with specific ligands would stop the peroxidase activity of cyt c/CL complex and lead to the inhibition of radiation-induced apoptosis. By considering the electrostatic and hydrophobic interactions in the cyt c, our group designed imidazole-substituted fatty acids, where imidazole might act as the sixth ligand to the heme iron.

During our initial studies we designed and synthesized (*S,Z*)-12-(1*H*-imidazol-1-yl)octadec-9-enoic acid (**IOA**), 12-(1*H*-imidazol-1-yl)octadecanoic acid (**ISA**), (*S,Z*)-methyl 12-(1*H*-imidazol-1-yl)octadec-9-enoate (**IEOA**) and 12-(1*H*-imidazol-1-yl)dodecanoic acid (**IDA**) (**Figure 6**).

These compounds were tested and shown to be able to inhibit cyt c peroxidase activity *in vitro* and to a lesser extent in isolated mitochondria. The compound **IDA** did not show any inhibitor activity against cyt c peroxidase activity *in vitro* and isolated mitochondria. Furthermore, **IOA**, **ISA** and **IEOA** could also stop the apoptotic process in cultured cells.

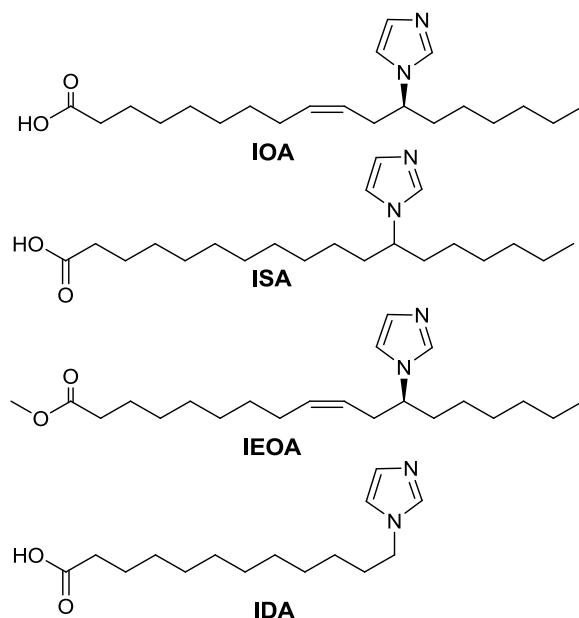


Figure 6: Structures of **IOA**, **ISA**, **IEOA** and **IDA**.⁴⁶

We designed and synthesized the **TPP-12-IOA** and **TPP-12-ISA** (**Figure 7**) to target the mitochondria, because of the delocalized cationic nature of triphenylphosphonium group effectively electrophoresed into the negatively charged mitochondrial membrane. **TPP-12-IOA** and **TPP-12-ISA** were tested in *in vitro* and *in vivo*, and worked both as inhibitors of cyt c peroxidase and radiation induced apoptosis.

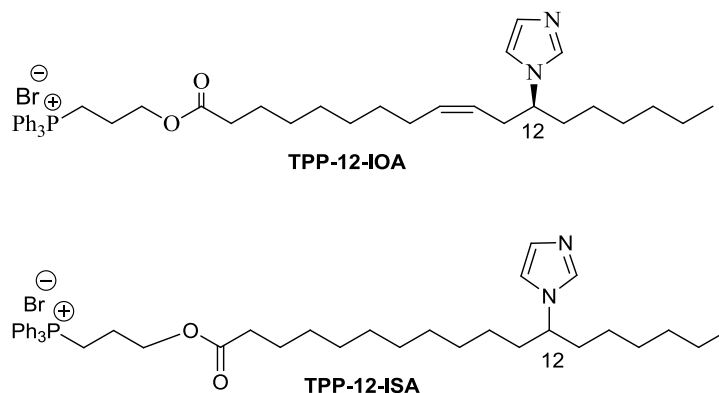


Figure 7: Structures of **TPP-12-IOA** and **TPP-12-ISA**.

Later, we were interested in studying the structure-activity relationship of molecules that have the imidazole moiety at different positions (6, 8, 10, 13 and 14) along the alkyl chain (**Figure 8**) on inhibiting the peroxidation activity of cytochrome c.

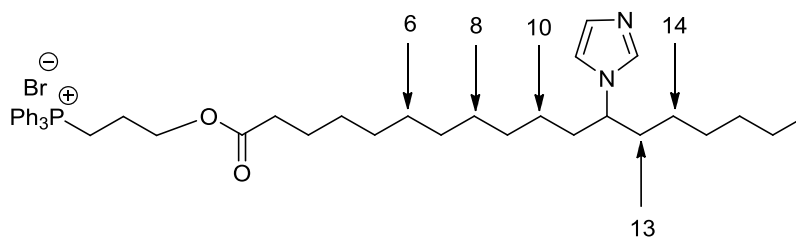


Figure 8: General scheme for different positional imidazole derivatives.

We designed and synthesized another group of molecules that are carnitine ester derivatives (**Figure 9**). Carnitine esters of fatty acids are part of the controlled mechanism of import of acyl chains into the mitochondria. We hypothesized that the attachment of carnitine esters may benefit the bio-compatibility and bio-availability of these compounds compared to the previously designed TPP derivatives. These molecules were tested for the ability to inhibit cyt c peroxidase activity *in vitro*.

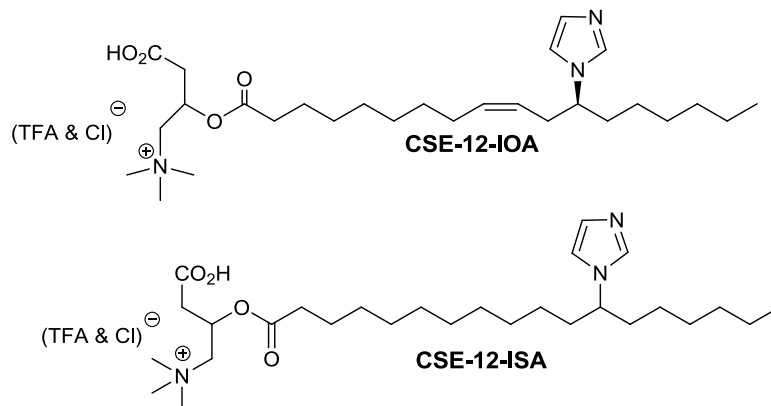


Figure 9: Structures of the carnitine esters **CSE-12-IOA** and **CSE-12-ISA**.

We designed and synthesized another set of compounds **CDE-12-IOA** and **CDE-12-ISA** (**Figure 10**). The double carnitine esters **CDE-12-IOA** and **CDE-12-ISA** are more hydrophobic and possess less detergent like properties than the single carnitine esters **CSE-12-IOA** and **CSE-12-ISA**.

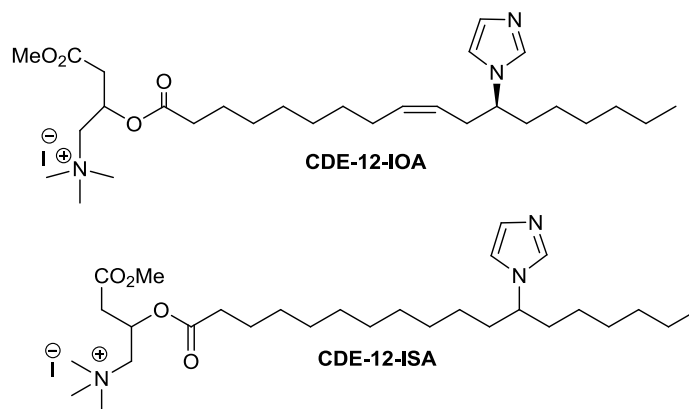


Figure 10: Structures of carnitine double esters **CDE-12-IOA** and **CDE-12-ISA**

2. Results and Discussion

2.1 Synthesis of positional isomers of imidazole substituted C18 fatty acids esters

Our main goal was the synthesis of different fatty acid derivatives containing an imidazole group on the alkyl chain and esterified to a triphenylphosphonium salt to direct the compound to mitochondria. Our previous group members synthesized imidazole-substituted fatty acids and their esters. Those were imidazole substituted oleic acid (IOA), stearic acid (ISA), imidazole substituted methyl ester of oleic acid (IEOA) and imidazole substituted dodecanoic acid (IDA) (**Figure 6**). They hypothesized that these compounds interact with cytochrome c to position the imidazole group as a sixth ligand to the heme.⁴⁶

Molecular modelling and docking studies to partially unfolded cyt c showed that imidazole group of IOA, ISA and IEOA close to heme. The distances between heme-iron and imidazole nitrogen were 2.4 Å (IOA), 2.5 Å (IEOA) and 2.7 Å (ISA). The imidazole group of compound IDA was 6.9 Å away from the heme-iron atom.⁴⁶ From the above data, it was confirmed that fatty acids with chains longer than C₁₂ can lie in a hydrophobic groove of partially unfolded cyt c. The distance between the imidazole group and heme iron plays a major role in the activity.

The Amplex Red reagent (10-acetyl-3,7-dihydroxyphenoxazine) is used to detect hydrogen peroxide (H₂O₂) (and thus indirectly peroxidase activity) in picomolar concentration. The Amplex Red reagent, in combination with horseradish peroxidase (HRP) is used to detect H₂O₂ released from biological samples, including cells, or generated in enzyme-coupled reactions. In the presence of HRP, the Amplex Red reagent reacts with H₂O₂ in a 1:1 stoichiometry to produce the red-fluorescent oxidation product, resorufin (**Figure 11**). Resorufin has absorption and

fluorescence emission maxima of approximately 571 nm and 585 nm, respectively. The assay for resorufin can be performed either fluorometrically or spectrophotometrically.

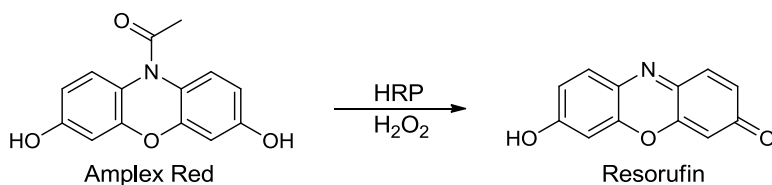


Figure 11: Oxidation of Amplex Red to resorufin.

Figure 12 shows the results of fluorescence Amplex Red based assay for the inhibitory activity of imidazole fatty acids and one ester against cyt c peroxidase. Here -TOCL is cyt c and H_2O_2 lacking 1,1',2,2' - tetraoleoyl cardiolipin (white bar) and + TOCL is cyt c and H_2O_2 plus 1,1',2,2' - tetraoleoyl cardiolipin (black bar). The compounds IEOA, IOA, ISA and IDA were tested against

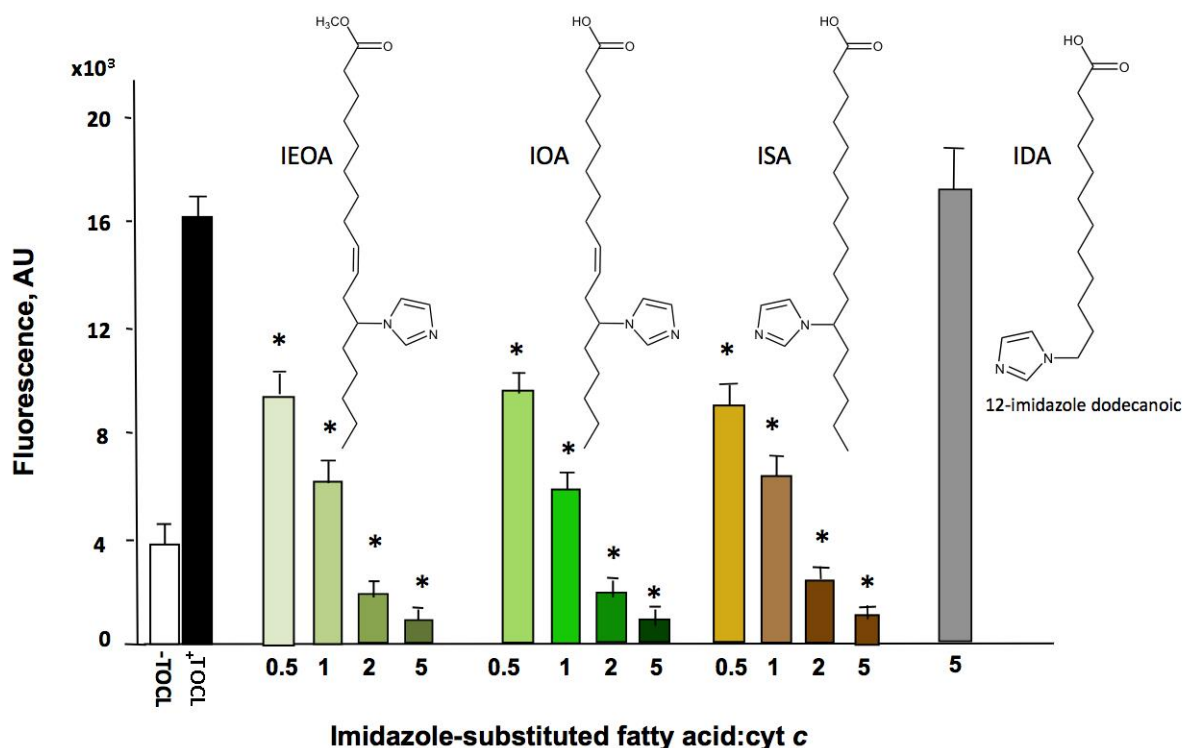


Figure 12: Inhibition of peroxidase activity of cyt c/TOCL complexes with IEOA, IOA, ISA, and IDA.

the peroxidase activity at different concentrations (0.5 to 5 μM). The inhibitory action on peroxidase activity increased upon increasing the concentrations of IEOA, IOA and, ISA compounds. On the contrary, the compound IDA was tested at 5 μM , but did not show any inhibition of the peroxidase activity of cyt c/cardiolipin complex.

Curiously, despite the anticipated requirement of the free carboxylate for binding to cyt c, the methyl ester also showed inhibitory activity. The 12-imidazole-dodecanoate analogue was not active, illustrating that the full C_{18} carbon chain of the fatty acid is necessary for inhibitory activity. The binding mode of the 12-IOA and 12-ISA to partially unfolded human cyt c was modeled by Drs. Judith Klein-Seetharaman and Naveena Yanamala, (Dept. of Structural Biology, University of Pittsburgh) and are shown in **Figure 13**.

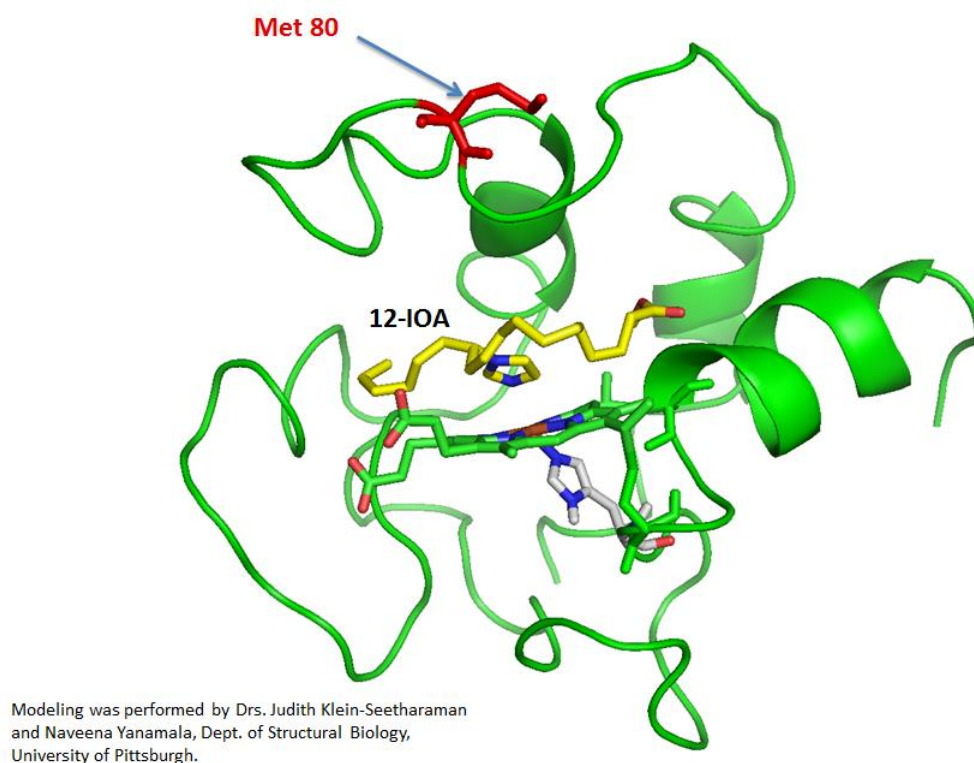


Figure 13: Docked modeling for the binding of IOA to cytochrome c

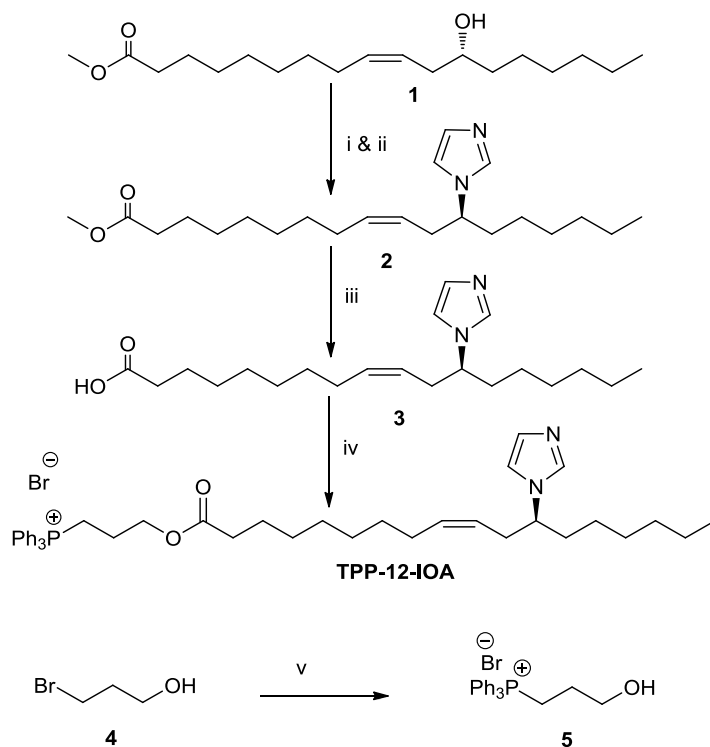
Free imidazole substituted fatty acids and esters inhibit the apoptosis in cells and targeted into mitochondria. The internal charge of mitochondria is negative and the inner membrane potential is 150-180 mV.⁵⁰ The large inner membrane potential should be used for delivering the molecules to mitochondria. Generally, the presence of the cationic charge on the delivering molecule favours the interaction with mitochondria. That is why lipophilic cations can pass easily through lipid bilayers. Here our group designed and synthesized imidazole fatty esters having the triphenylphosphonium group. The triphenylphosphonium group increases the cationic surface which can assist in interacting with mitochondrial membrane. These molecules enter into the cytoplasm from the extracellular environment driven by the plasma membrane potential (30-60 mV) and again accumulated into mitochondria.^{50,51} Most of TPP derivatives accumulated in mitochondria⁴⁶ and inhibited apoptosis in cells, similarly to **IOA** and **ISA**. Due to this reason, our group synthesized the **TPP-12-IOA** and **TPP-12-ISA** compounds.

We synthesized different positional isomers of the imidazole ring on the long carbon chain, at positions 6, 8, 10, 13 and 14. Also prepared were C₁₂ (saturated and unsaturated) imidazole carnitine monoesters as well as double esters. Compounds were sent to the Centre for Medical Countermeasures against Raditation (CMCR) at the University of Pittsburgh for biological testing. In order to prepare these compounds we started with different hydroxy fatty acids, some of which are commercially available from natural sources and some which had to be prepared synthetically. Syntheses of all these compounds are shown in the corresponding schemes.

2.2.1 Synthesis of (*S, Z*)-(3-((12-(1*H*-imidazol-1-yl)octadec-9-enoyl)oxy)propyl)triphenylphosphonium bromide (TPP-12-IOA).

TPP-12-IOA and TPP-12-ISA were synthesised from the commercially available starting material methyl ricinoleate. Both compounds were synthesized in similar manner (**Scheme 1**).

Methyl ricinoleate **1** was mesylated by treating with methanesulfonyl chloride, in the presence of TEA and DMAP in DCM.⁵² The crude mesylated compound was heated with imidazole at 70°C to give the imidazole substituted compound **2** in 34% yield over the two steps.



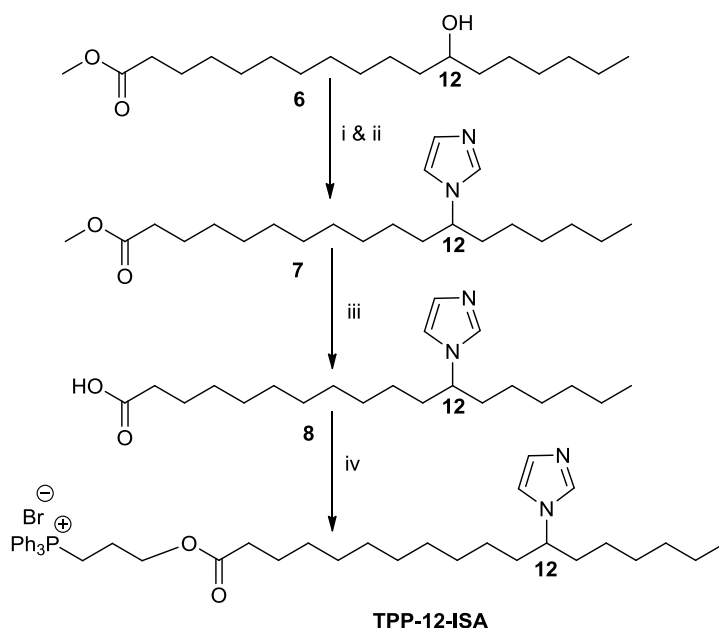
Reaction Conditions: (i) CH₃SO₂Cl, TEA, DMAP, DCM, 0°C to RT; (ii) imidazole, 70°C, 16h, 34%; (iii) MeOH : H₂O (3:1), NaOH, RT, 89%; (iv) Compound 5, DCC, DCM, 85%; (v) TPP, toluene, 110°C, 16h, 95%.

Scheme 1: Synthesis of TPP-12-IOA.

We observed that most of the compound was the product of elimination because the secondary mesyl group is likely prone to this reaction. We used higher temperatures in the presence of

imidazole, and the pKa values of imidazole are 7 and 14.2⁵³. Hydrolysis of the imidazole methyl ester **2** with NaOH in a mixture of methanol and water gave acid **3** in 89% yield.⁵⁴ Compound **4** was refluxed with TPP in toluene to produce the compound **5** in 95% yield.^{55,56} Compound **3** was esterified with (3-hydroxypropyl) triphenylphosphonium bromide **5** in the presence of DCC to give compound **TPP-12-IOA** in 85% yield.⁵⁷

2.2.2 Synthesis of (3-((12-(1*H*-imidazol-1-yl)octadecanoyl)oxy)propyl)triphenylphosphonium bromide (TPP-12-ISA)



Reaction Conditions: (i) CH₃SO₂Cl, TEA, DMAP, DCM, 0°C to RT.; (ii) imidazole, 70°C, 16h, 34%; (iii) MeOH : H₂O (3:1), NaOH, RT, 89%; (iv) Compound 5, DCC, DCM, 85%.

Scheme 2: Synthesis of TPP-12-ISA.

Synthesis of **TPP-12-ISA** was started with 12-hydroxy stearic acid methyl ester and was prepared in an analogous fashion to **TPP-12-IOA**. The strategy for the synthesis of compound **TPP-12-ISA** was shown in the above **Scheme 2**.

2.2.3 Biological results of TPP-12-IOA and TPP-12-ISA

TPP-12-IOA and **TPP-12-ISA** were both tested in *in vitro* and *in vivo* as inhibitors of cyt c peroxidase and as inhibitors of radiation induced apoptosis. **Figure 14** shows the survival of mice that had been given doses of the compounds either before or directly after receiving a dose of radiation that is lethal to most of the animals by day 12 post-irradiation. For this experiment they used the three sets of mice that were observed over 60 days.

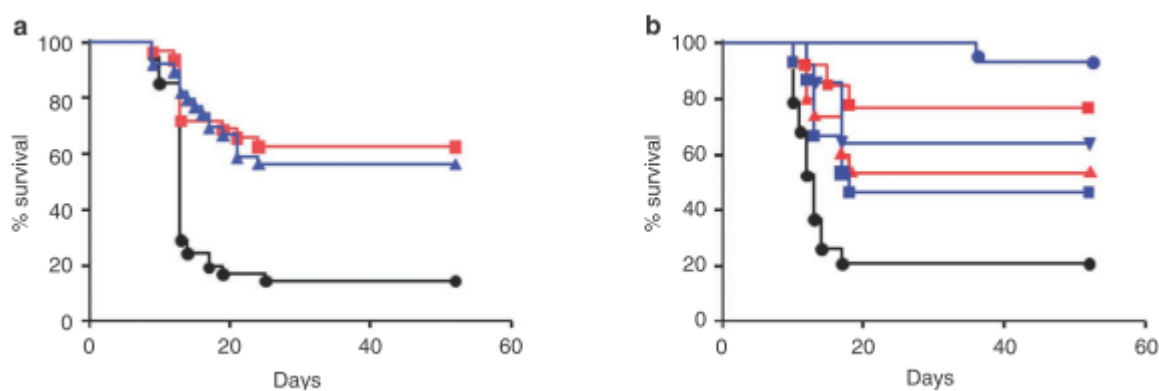


Figure 14: Radiation protection and mitigation by **TPP-12-IOA** and **TPP-12-ISA**.⁴⁸

C57BL/6NTac female mice were exposed to total body irradiation to a dose of 9.25 Gy using (a) a cesium source ($n = 31\text{--}35$ mice per group) (b) a linear accelerator ($n = 10\text{--}23$ mice per group). The mice were irradiated and injected intraperitoneally (i.p.) with TPP-12-IOA or TPP-12-ISA (5 mg per kg body weight in 100 μ l of water containing 25% ethanol) 10 min after irradiation. (i) The first set mice were exposed to total body irradiation at the dose of 9.25 Gy only (black circles). (ii) The second set mice were exposed to total body irradiation at the dose of 9.25 Gy and injected with TPP-12-ISA (5 mg per kg body weight) 10 min (blue triangles) (iii) The third set of mice were exposed to total body irradiation at the dose of 9.25 Gy and injected with TPP-12-IOA (5 mg per kg body weight) 10 min (red squares).

Survival of the mice was monitored for 55 days. Most of the untreated mice (~75%) were dead after 15 days (black circles), and ~15% of mice survived from 25 to 55 days. Mice that were treated with TPP-12-IOA after radiation, showed 90% survival up to 15 days, and after that suddenly dropped to 70%, then after 25 days about 65% survived. In a third set of animals, ~90% of the mice that were treated with TPP-12-ISA were still alive at 10 days, 85% at 15 days and 55% from day 25 onward. In the same way they tested again three more sets of mice with different conditions and their results are shown in the **Figure 14b**.

The conditions are the same as in (a) but mice were injected with the compounds before and after irradiation. The mice were injected i.p. with TPP-12-IOA (5 mg per kg body weight in 100 μ l of water containing 25% ethanol) at 1 h or 10 min before irradiation or 10 min, 5 or 24 h after irradiation. Mice were exposed to total body irradiation at the dose of 9.25 Gy only (black circles); to total body irradiation at the dose of 9.25 Gy and injected with TPP-12-IOA (5 mg per kg body weight) 10 min (blue circles), 5 h (blue triangles) and 24 h (blue squares) thereafter. Mice injected with TPP-12-IOA (5 mg per kg body weight) 10 min (red squares) and 1 h (red triangles) before total body irradiation (9.25 Gy).

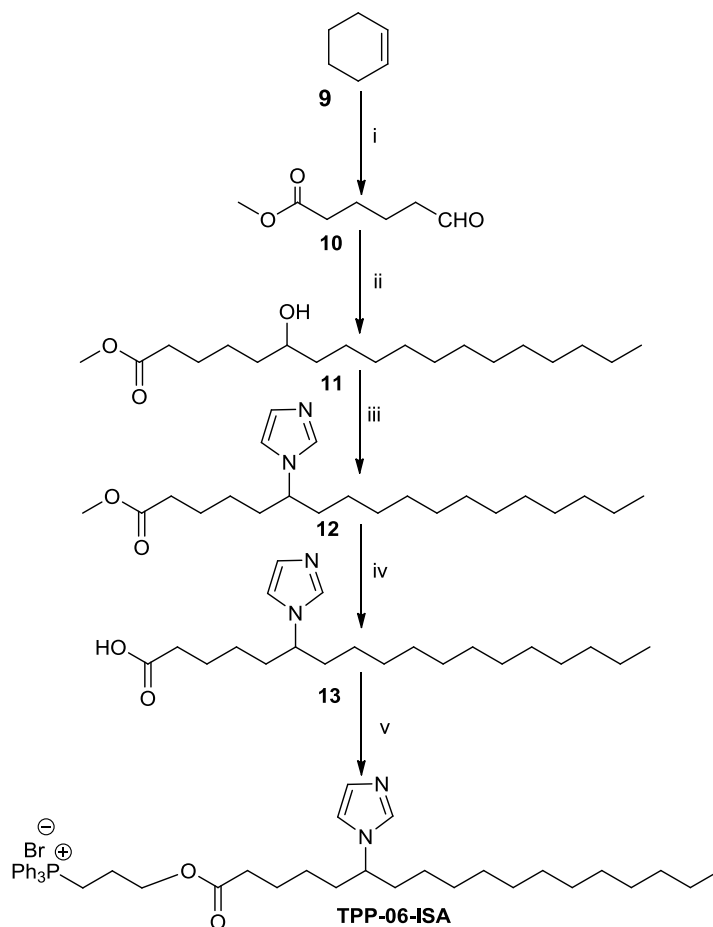
In the **Figure 12 (b)**, those animals that received injections of TPP-12-IOA ten minutes after irradiation (blue circles) showed remarkable survival rates; 100% up to 40 days, then 95% up to 55 days.

2.3.1 Synthesis of (3-((6-(1*H*-imidazol-1-yl)octadecanoyl)oxy)propyl)triphenylphosphonium bromide (TPP-6-ISA)

TPP-12-IOA and TPP-12-ISA were tested *in vitro* and *in vivo* and were shown to be active as inhibitors of cyt c peroxidase. It was not obvious from the preliminary assay and modeling results whether imidazole substitution at other places on the chain would decrease or increase inhibitory activity. We therefore planned to synthesize different positional isomers of imidazole on the alkyl chain, to study the structure activity relationship of TPP-imidazole fatty acids in an attempt to identify more potent compounds. TPP-12-ISA and TPP-12-IOA were made by using commercially available 12-hydroxy steric acid and ricinoleic acid. The 6, 8, 10, 13 and 14-hydroxy fatty acids were synthesized by using different starting materials because these compounds are not commercially available. 6 and 8-Hydroxy fatty acids were synthesized from cycloalkenes. The 10-hydroxy fatty acid can also be prepared by using this method, but cyclodecene is more expensive. Because of this reason, 10-hydroxy fatty acid was prepared from 1, 10-decanediol. The 13 and 14 hydroxy fatty acids were also synthesized from 1, 12-dodecanediol.

In general, ozonolysis of olefins gives the dialdehydes but conditions exist that produce a methyl ester and an aldehyde. Starting from a cyclic alkene would therefore provide a differentially terminally functionalized linear alkyl chain. Cleavage of cyclohexene **9** by passing ozone gas at -78°C through a suspension of compound **9** and NaHCO₃ in DCM, followed by the addition of Ac₂O and TEA for 15 minutes produced the aldehyde **10** in 63% yield.^{58, 59} Compound **10** in THF was added to dodecylmagnesium bromide at -20°C, which was prepared from 1-bromododecane and magnesium metal in THF, to give the alcohol **11** in 47% yield. This

alcohol **11** was converted into the mesylate, and the mesyl group was displaced with imidazole to provide compound **12** in 34% yield.

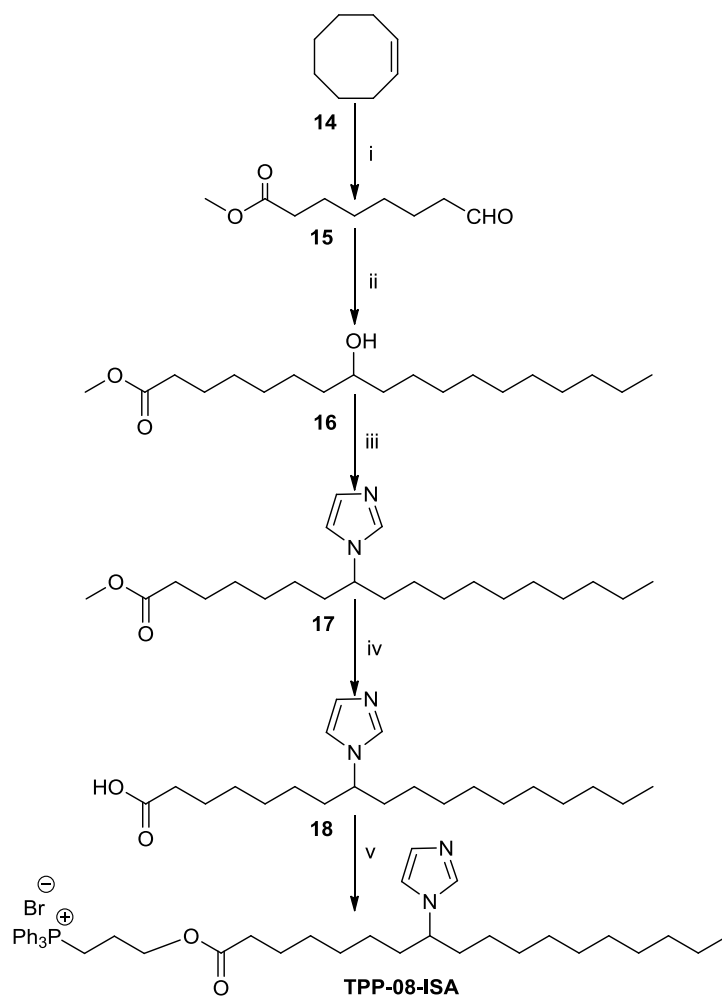


Reaction Conditions: (i) NaHCO_3 , ozone gas, -78°C , DCM, TEA, Ac_2O , DCM, 16h, 63%; (ii) 1-bromododecane, Mg, THF, -20°C , 3h, 47%; (iii) $\text{CH}_3\text{SO}_2\text{Cl}$, TEA, DMAP, DCM, 0°C to RT, Imidazole, 70°C , 16h, 34%; (iv) MeOH : H_2O (3:1), NaOH, RT, 3h, 77%; (v) Compound 5, DCC, DCM, 16h, 67%.

Scheme 3: Synthesis of TPP-6-ISA.

Hydrolysis of compound **12** in the presence of a base gives carboxylic acid **13** in 77% yield. The carboxylic acid **13** was coupled with phosphonium salt **5** to produce **TPP-6-ISA** in 67% yield. The last three steps were the same as those for the previous compounds **TPP-12-IOA** and **TPP-12-ISA** (Scheme 1).

2.3.2 Synthesis of (3-((8-(1H-imidazol-1-yl)octadecanoyl)oxy)propyl)triphenylphosphonium bromide (TPP-8-ISA)



Reaction Conditions: (i) a) Cyclohexene, NaHCO_3 , ozone gas, -78°C , DCM, TFA, Ac_2O , DCM, 16h, 70%; (ii) 1-bromododecane, Mg, THF, -20°C , 2h, 47%; (iii) $\text{CH}_3\text{SO}_2\text{Cl}$, TEA, DMAP, DCM, 0°C to RT, Imidazole, 70°C , 16h, 35%; (iv) MeOH : H_2O (3:1), NaOH, RT, 83%; (v) Compound 5, DCC, DCM, 70%.

Scheme 4: Synthesis of TPP-8-ISA.

Compound **TPP-8-ISA** was synthesized by using the procedures of compound **TPP-6-ISA** but yields were little bit different from previous compound **TPP-6-ISA**. All the yields and conditions are shown in **Scheme 4**.

2.3.3 Biological results of compounds TPP-6-ISA and TPP-8-ISA

Compounds **TPP-6-ISA** and **TPP-8-ISA** were tested and found to be competent inhibitors of cytochrome c peroxidase in *in vitro*. Based on these results we decided to study the structure activity relationship of different positional isomers of TPP-imidazole fatty acids to potentially identify more potent compounds. The aim is to create a robust structure-function study of these compounds to guide the choice of the best compound for further studies in mice. Compounds with the imidazole group substituted at C-6 or C-8 were synthesized and sent to our collaborators at the Centre for Medical Countermeasures against Radiation (CMCR) at the University of Pittsburgh.

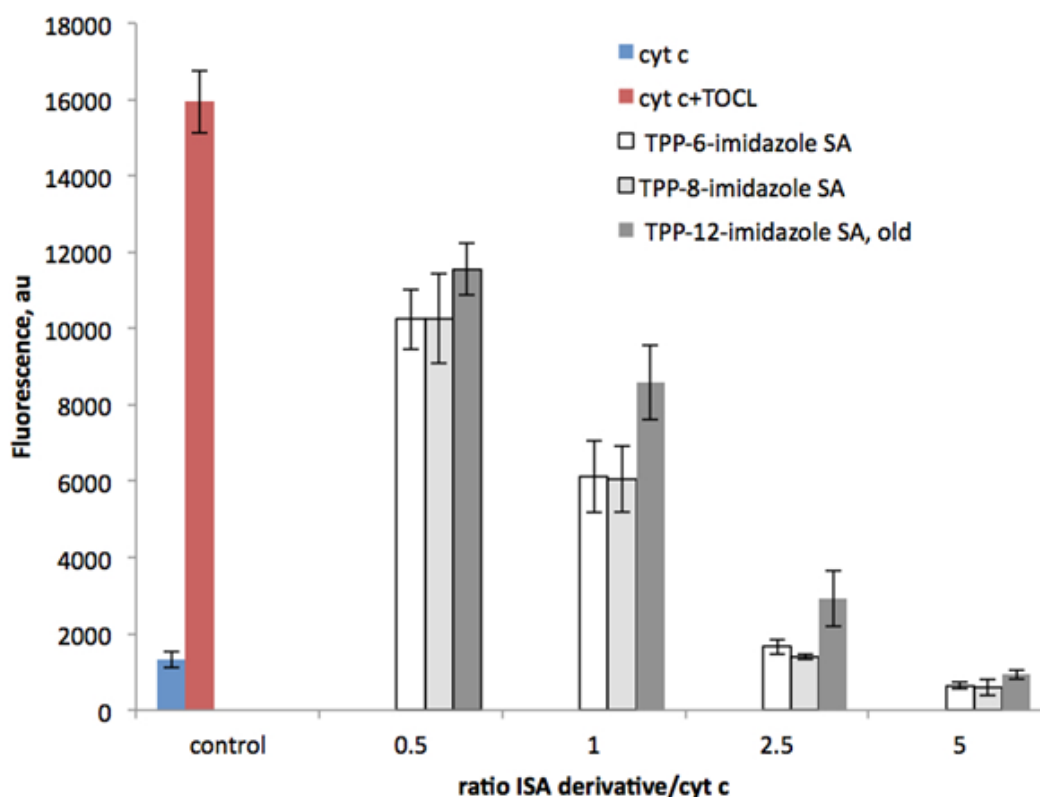


Figure 15: TPP-imidazole-stearic acid derivatives (TPP-ISAs) inhibit peroxidase activity of cytochrome c/TOCL complexes. New derivatives: TPP-6-ISA and TPP-8-ISA, and TPP-12-ISA.

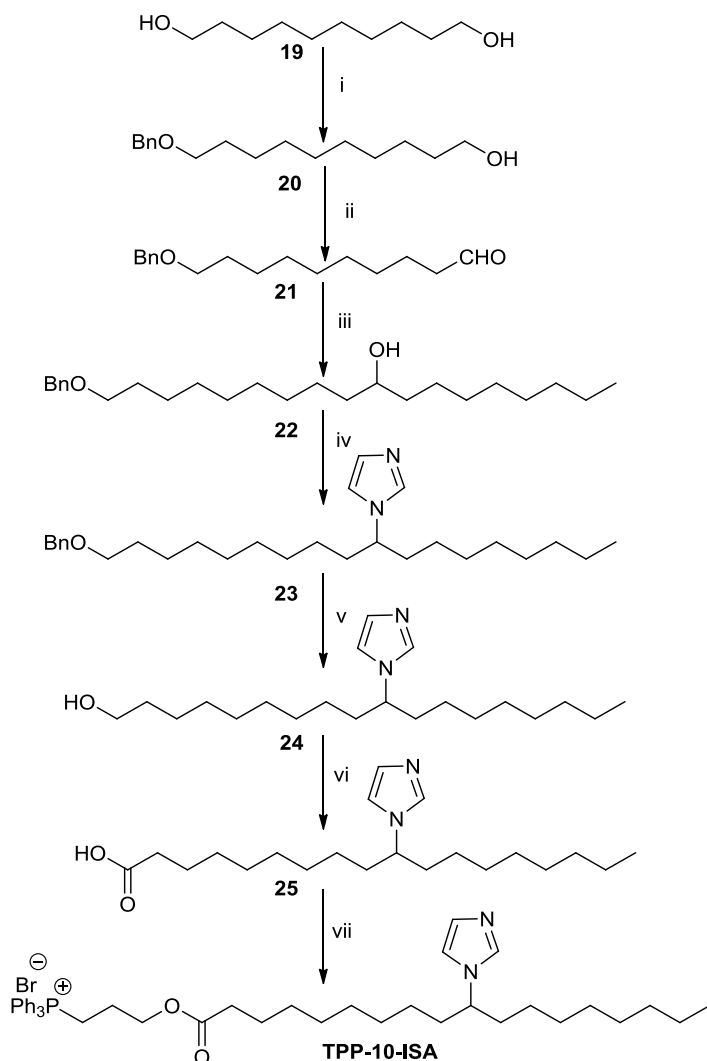
Conditions: buffer 20 mM HEPES (pH 7.4) with 100 μ M DTPA, 1 μ M cytochrome c 50, μ M Amplex Red, 50 μ M H_2O_2 .

In **Figure 15** blue and red colored bars indicate fluorescence intensity of cyt c and cyt c + TOCL respectively. The cyt c/TOCL complex showed the maximum peroxidase activity. The inhibitory studies were performed on 6-, 8- and 12-imidazole TPP stearates at various concentrations. At lower and higher concentration (0.5 μ M and 5 μ M) for all three compounds, inhibitory action against peroxidase activity was found to be very similar. But at moderate concentration (1 μ M and 2.5 μ M) **TPP-6-ISA** and **TPP-8-ISA** appeared to be significantly better inhibitors compared to the **TPP-12-ISA**.

2.4 Synthesis of (3-((10-(1*H*-imidazol-1-yl)octadecanoyl)oxy)propyl)triphenylphosphonium bromide (TPP-10-ISA)

The preparation of compound **TPP-10-ISA** is shown in **Scheme 5**. The 10-hydroxy fatty acid starting material is not commercially available. **TPP-10-ISA** can be synthesized by the ozonolysis of cyclodecene. But this starting material is very expensive. Due to this reason, the synthesis was started with decane-1,10-diol **19** which was treated with benzyl bromide and Ag₂O in DCM at reflux for 16 h to give the mono protected compound **20** in 65% yield.⁶⁰ Compound **20** was oxidized with PCC in DCM⁶¹ to produce the aldehyde **21** in 78% yield. The bromooctane was first converted to a Grignard reagent with Mg metal and iodine in dry THF, and the solution so prepared was added to the aldehyde **21** in THF solution at 0°C to give the compound **22** in 61% yield. Mesylation of hydroxyl compound **22** was achieved with methanesulfonyl chloride, TEA and DMAP in DCM, which was substituted with imidazole by heating without solvent at 70°C for 16h to give the compound **23** in 33% yield. Compound **23** was debenzylated with 10% palladium on carbon in ethanol under hydrogen gas balloon at reflux for two days to produce

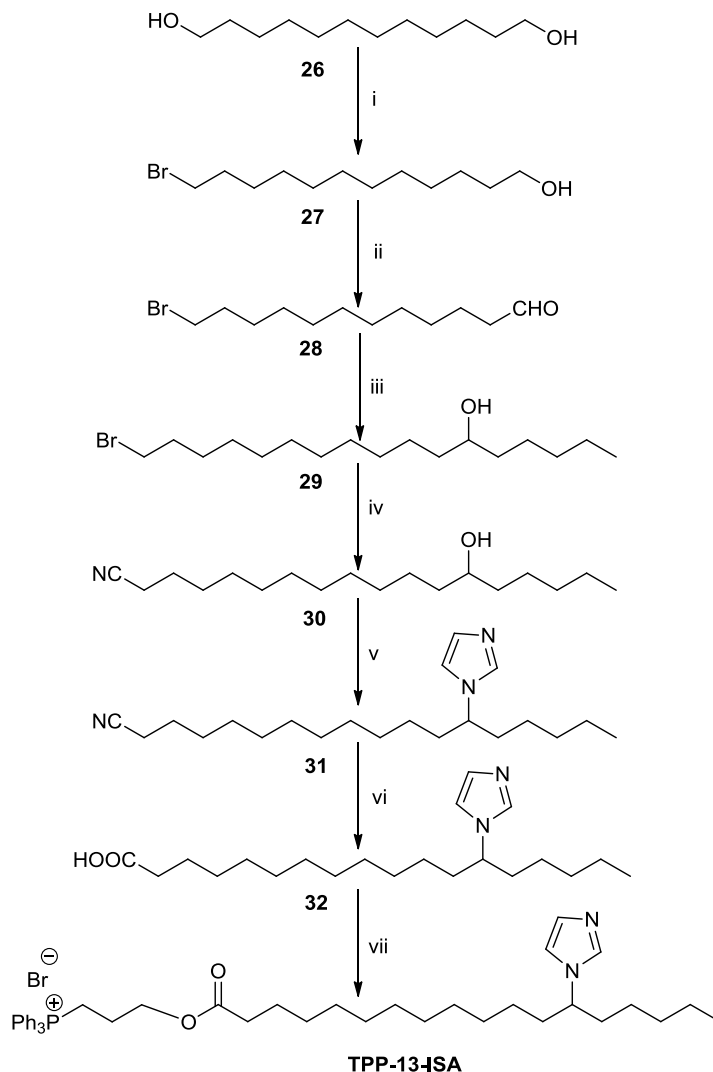
hydroxyl compound **19** in 81% yield. Compound **19** was converted into acid **25**⁶² with freshly prepared Jones reagent in acetone water mixture for 3 hours in 84% yield. The acid **25** was esterified with compound **5** by using DCC, DMAP in DCM to produce TPP-10-ISA in 77% yield.



Reaction Conditions: (i) BnBr, Ag₂O, DCM, reflux, 18h, 65%; (ii) PCC, DCM, 4h, 78%; (iii) Bromooctane, Mg, dry THF, 4h, 61%; (iv) a) CH₃SO₂Cl, TEA, DMAP, DCM, 2h; b) Imidazole, 16h, 70°C, 32.5%; (v) 10% Pd-C, EtOH, reflux, 2 days, 81%; (vi) Jones reagent, Acetone, 3h, 84%; (vii) compound **5**, DCC, DCM, 16h, 77%.

Scheme 5: Synthesis of **TPP-10-ISA**

2.4.1 Synthesis of (3-((13-(1*H*-imidazol-1-yl)octadecanoyl)oxy)propyl)triphenylphosphonium bromide (TPP-13-ISA)



Reaction Conditions: (i) 48% HBr in H₂O, Toluene and reflux, 3h, 78%; (ii) PCC, DCM, 4h, 72%; (iii) 1-bromopentane, Mg, THF, 2h, 58%; (iv) NaCN, DMF, 16h, 92% (v) CH₃SO₂Cl, TEA, DMAP, DCM, 0°C to RT, 2h, Imidazole, 70°C, 16h, 33%; (vi) NaOH, EtOH, H₂O, reflux, 81%; (vii) Compound 5, DCC, DCM, 78%;

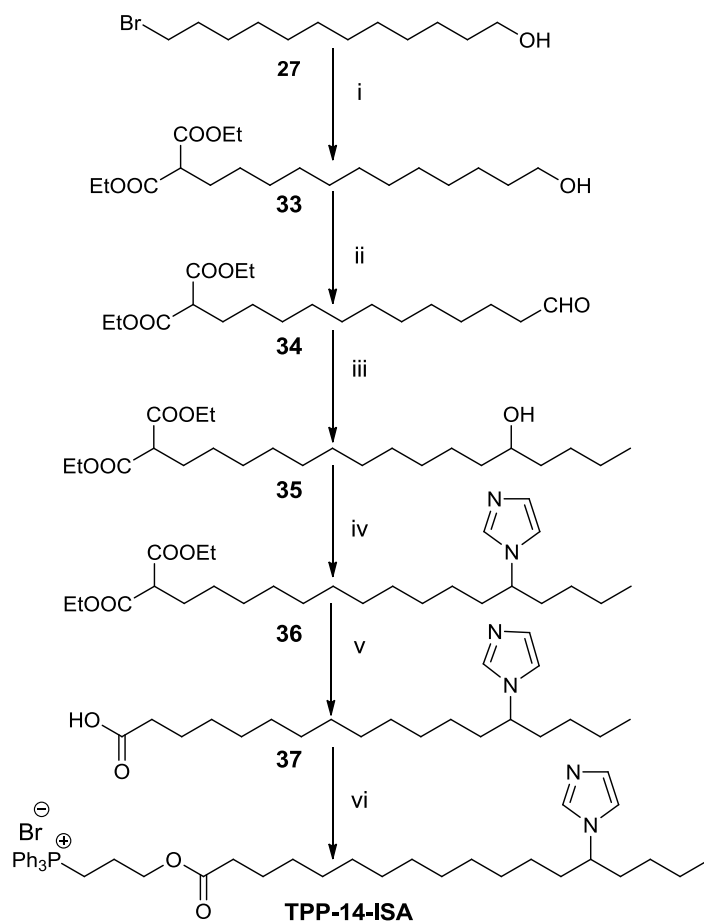
Scheme 6: Synthesis of TPP-13-ISA

Compound **TPP-13-ISA** (Scheme 6) was prepared from 1,12-dodecanediol **26** by mono bromination with 45% HBr in water and toluene at reflux for 3 hours to give compound **27** in

78% yield.⁶³ Oxidation of alcohol **27** with PCC in DCM for 4h produced the aldehyde **28** in 72% yield. Pentyl magnesium bromide was prepared from bromopentane using magnesium metal in THF, which was added to compound **28** to give the alcohol **29** in 58% yield. The extension of one carbon on bromoalcohol **29** was achieved by the reaction between compound **29** and NaCN in DMF at 90°C to give compound **30** in 92% yield.⁶⁴ Compound **30** was mesylated followed by imidazole substitution to give the compound **31** in 32% yield. Hydrolysis of compound **31** with NaOH in ethanol at reflux for 16h gave the acid **32** in 81% yield.⁶⁵ Finally, the acid **32** was esterified with compound **5** using DCC and DMAP in DCM to produce the final compound **TPP-13-ISA** in 78% yield as a gummy liquid.

2.4.2 Synthesis of (3-((14-(1*H*-imidazol-1-yl)octadecanoyl)oxy)propyl)triphenylphosphonium bromide (TPP-14-ISA)

Synthesis of **TPP-14-ISA** (**Scheme 7**) was started with compound **27** which was prepared in previous **Scheme 6**. Our goal was to extend the fatty acid chain by two carbon atoms on. It is possible by the substitution of bromo compound **27** with diethyl malonate. So the bromo compound **27** was treated with diethyl malonate in the presence of base and KI at reflux conditions to produce the compound **33** with extension of two carbon atoms in 63% yield.⁶⁶ Compound **33** was treated with PCC to produce the aldehyde **34** in 73% yield. The produced aldehyde **34** was treated with Grignard reagent to give the secondary alcohol **35** in 63% yield. Compound **35** was mesylated and substituted with imidazole at 70°C to produce the compound **36** in 33 % yield.



Reaction Conditions: (i) Diethyl malonate, K_2CO_3 , KI, Acetonitrile, 90°C , 16h, 63%; (ii) PCC, DCM, 3h, 73%; (iii) 1-bromobutane, Mg, dry THF, 2h, 63%; (iv) $\text{CH}_3\text{SO}_2\text{Cl}$, TEA, DMAP, DCM, 0°C to RT.; imidazole, 70°C , 16h, 33%; (iii) MeOH : H_2O (3:1), NaOH, RT, 170°C , 15min, 68%; (iv) Compound 5, DCC, DCM, 71%.

Scheme 7: Synthesis of TPP-14-ISA

Hydrolysis followed by mono decarboxylation of diester **36** produced the mono acid **37** in 68 % yield.⁶⁶ The mono acid **37** was esterified with compound **5** and **DCC** to obtain compound **TPP-14-ISA** in 71% yield.

2.4.3 Biological results of compounds TPP-6-ISA, TPP-8-ISA, TPP-10-ISA, TPP-12-ISA, TPP-13-ISA and TPP-14-ISA

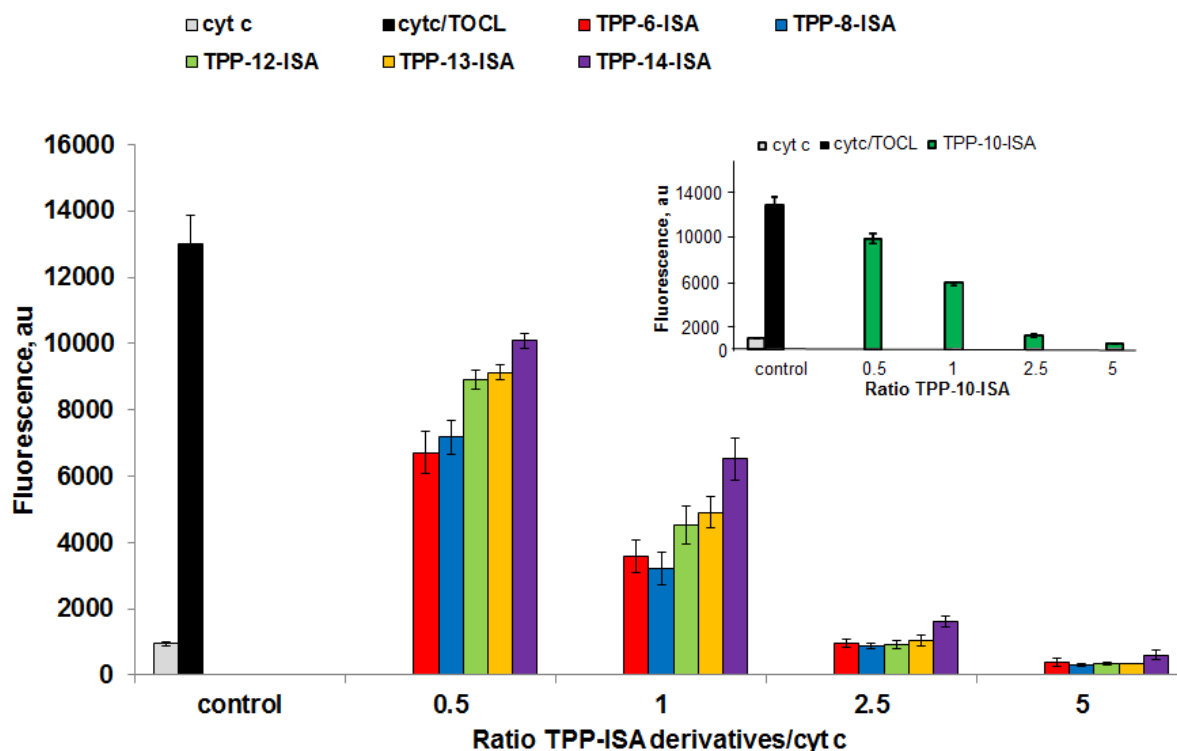


Figure 16: Effect of TPP- imidazole-stearic acid (TPP-ISA) derivatives on peroxidase activity of cyt c/TOCL complexes assessed by H₂O₂-dependent oxidation of Amplex Red.

Conditions: cyt c (1 μ M), H₂O₂ (50 μ M), Amplex Red (50 μ M). Experiments were performed in 20 mM HEPES buffer (pH 7.4) containing DTPA (100 μ M), cyt c was incubated with DOPC/TOCL liposomes (1:1) at ratio TOCL/cyt c 25:1.

Insert: Effect of TPP-10-ISA on peroxidase activity of cyt c/TOCL complexes.

In **Figure 16** the grey and black colored bars represent fluorescence intensity of cyt c and cyt c/TOCL complex respectively. TPP imidazole stearic acids conjugates (6, 8, 10, 12, 13 and 14) were tested against the peroxidase activity *in vitro* by using Amplex Red as quantitative fluorescent indicator. The conjugates showed similar activity at higher concentrations (2.5 μ M and 5 μ M), but at lower concentrations (0.5 μ M and 1 μ M), TPP-6-ISA and TPP-8-ISA showed higher inhibitory action compared to other derivatives. The compound TPP-14-ISA at different

concentrations (0.5 μM to 5 μM) showed the lowest inhibition against peroxidase activity compared to other conjugates.

2.5.1 Synthesis of Carnitine esters of IFAs

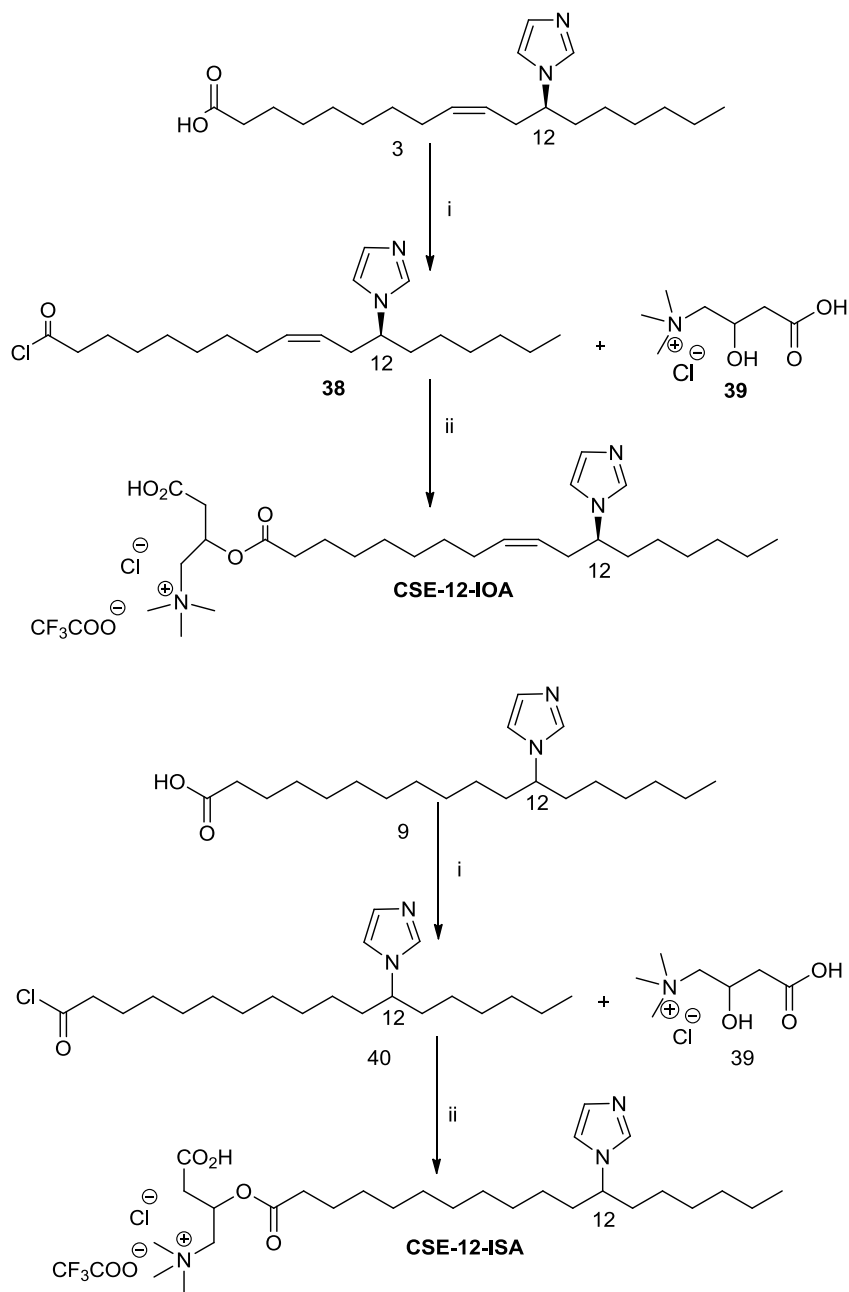
Within the cell, import of fatty acids into mitochondria is strictly controlled. Free fatty acids are in very low concentration in most cells, occurring rather as esters with co-enzyme A or bound to binding and transfer proteins.⁶⁷

Mainly three proteins are involved in this transport system carnitine palmitoyltransferase I (CPT-I), carnitine acylcarnitine translocase (CACT) and carnitine palmitoyltransferase II (CPT-II). Each one of these proteins is located in different regions of mitochondria. Acyl-CoAs are synthesized by the catalytic action of long chain acyl-CoA synthetase (LCAS) in the mitochondrial outer membrane (MOM), then are transformed to acylcarnitines. CPT-I catalyzed this transesterification in the MOM. The formed long-chain acylcarnitines are transferred into the mitochondrial matrix by an exchange reaction catalyzed by CACT, an integral inner membrane protein. The acylcarnitines are then exchanged to the corresponding acyl-CoAs within the matrix by CPT-II, an enzyme associated with the inner leaflet of the mitochondrial inner membrane (MIM).⁶⁷

We synthesized carnitine esters of IFAs as these may also allow selective drug import into mitochondria by a more specific means than did the TPP conjugates.

2.5.2 Synthesis of mono(2-(((S,Z)-12-(1H-imidazol-1-yl)octadec-9-enoyl)oxy)-3-carboxy-N,N,N-trimethylpropan-1-aminium) mono(2,2,2-trifluoroacetate) monochloride (CSE-12-

IOA) and mono(2-((12-(1*H*-imidazol-1-yl)octadecanoyl)oxy)-3-carboxy-*N,N,N*-trimethylpropan-1-aminium) mono(2,2,2-trifluoroacetate) monochloride (CSE-12-ISA)



Reaction Conditions: (i) Oxalyl chloride, DMF, DCM, (ii) Carnitine, TFA, 45⁰C, 75%

Scheme 8: Synthesis of **CSE-12-IOA** and **CSE-12-ISA**

Both carnitine esters **CSE-12-IOA** and **CSE-12-ISA** were prepared and purified by the same process. First, imidazole fatty acids **3** and **9** were converted into acid chlorides **38** and **40** with oxalyl chloride in DCM separately, and then these prepared acid chlorides were added to carnitine hydrochloride in TFA and heated at 50°C to produce the carnitine esters **CSE-12-IFA** and **CSE-12-ISA** in 75% yield.^{68,69} **CSE-12-IFA** and **CSE-12-ISA** were purified by precipitation method. These pure compounds were obtained as TFA salts. We tried to convert TFA salts into HCl salts by using 6N HCl in dioxane. These TFA salts were dissolved in 1,4-dioxane, then 6N HCl was added to it and concentrated under reduced pressure. This process was repeated for 3 times to get **CSE-12-IFA** and **CSE-12-ISA** as HCl salts. But, **CSE-12-IFA** and **CSE-12-ISA** were obtained as the 1:1 ratio of TFA and HCl salts.

2.5.3 Biological results of CSE-12-IOA (12-CIOFA) and CSE-12-ISA (12-CISFA).

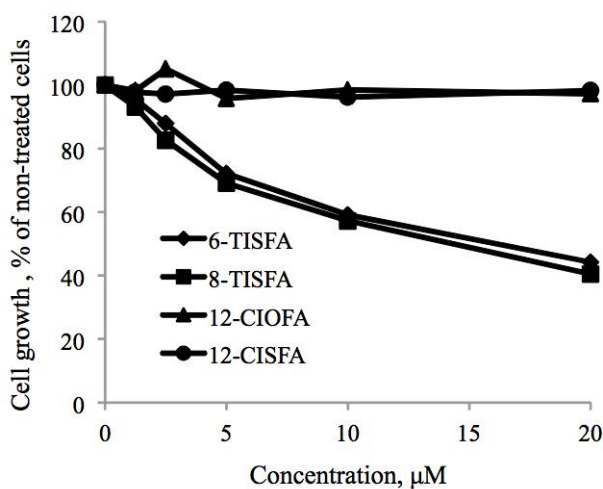


Figure 17: Cytotoxicity of TPP or carnitine conjugated fatty acid in mouse embryonic cells.

Cells were seeded in 24-well plate at a density of 0.02×10^6 /well and allowed to attach overnight.

Cells were then incubated with fatty acid conjugates at the indicated concentration (1.25, 2.5, 5,

10 and 20 μM) for 48 hours. The cytotoxicity of fatty acid conjugates were assessed by annexin V/propidium iodide assay (flow cytometry) or Alarma blue assay (fluorescent spectrophotometry). (Data provide by the CMCR)

Figure 17 shows that 12-CIOFA and 12-CISFA had very minimal detrimental effect at different concentrations on cell growth on non-treated cells, compared to 6- and 8-imidazole TPP derivatives which were shown to have concentration dependent growth inhibition of cells beyond a few micromolar concentration.

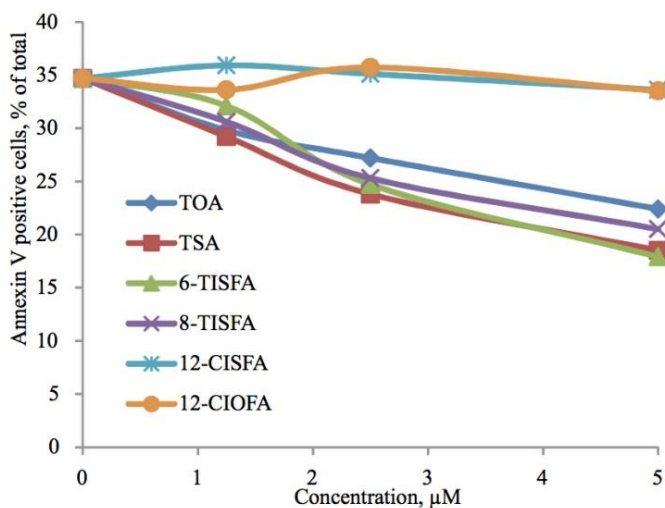


Figure 18: Radiomitigative effect of TPP or carnitine conjugated fatty acid in mouse embryonic cells. Cells were seeded in 6-well plate at a density of $0.05 \times 10^6/\text{well}$ and allowed to attach overnight. Fatty acid conjugates were added to cells 15 min after gamma-irradiation exposure (10 Gy). Irradiation induced cell death was assessed by Annexin V/Propidium iodide staining assay using flow cytometry after 48 hours incubation. Data presented are the Annexin V positive cells. (Data provide by CMCR)

Figure 18 shows that 12-CIOFA and 12-CISFA did not exhibit radiomitigative effects at concentrations ranging from 1 - 5 μM , whereas the 6- and 8-imidazole TPP derivatives showed a concentration dependent (1 μM to 5 μM) increase in radiomitigative effects, as assessed by a decreasing degree of annexin V positive cells, a marker for apoptosis.

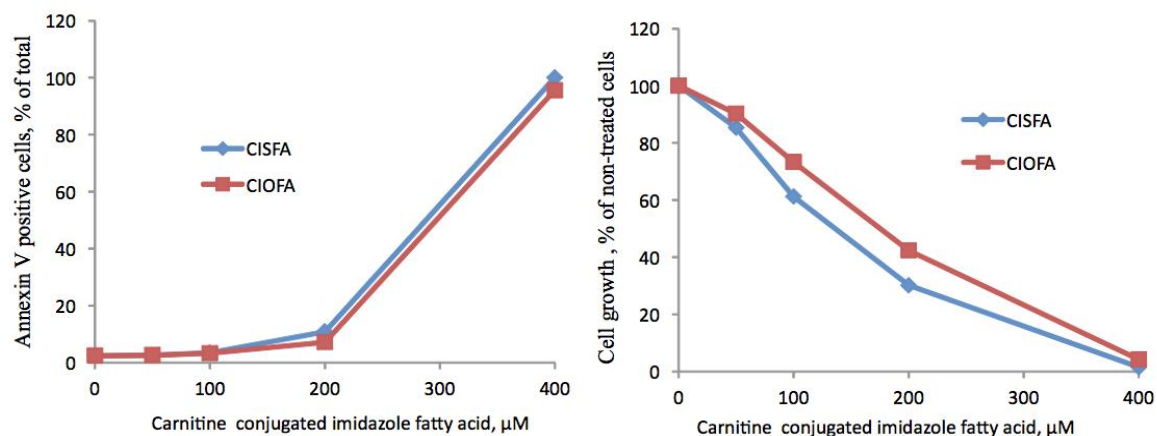


Figure 19: Cytotoxicity of carnitine conjugated imidazole fatty acids (CISFA and CIOFA) in mouse embryonic cells. Cells were seeded in 24-well plate at a density of $0.015 \times 10^6/\text{well}$ and allowed to attach overnight. Cells were then incubated with fatty acid conjugates at the indicated concentration (50, 100, 200 and 400 μM) for 48 hours. The cytotoxicity of fatty acid conjugates were assessed by annexin V/propidium iodide assay (flow cytometry) or Alarma blue assay (fluorescent spectrophotometry). (Data provided by CMCR)

Figure 19 shows that the cytotoxic effects of 12-CIOFA and 12-CISFA are concentration dependent beginning at $\sim 100 \mu\text{M}$. At 400 μM the compounds are highly toxic to the cells. Compounds 12-CIOFA and 12-CISFA have also shown to reduce the cell growth at increasing concentrations.

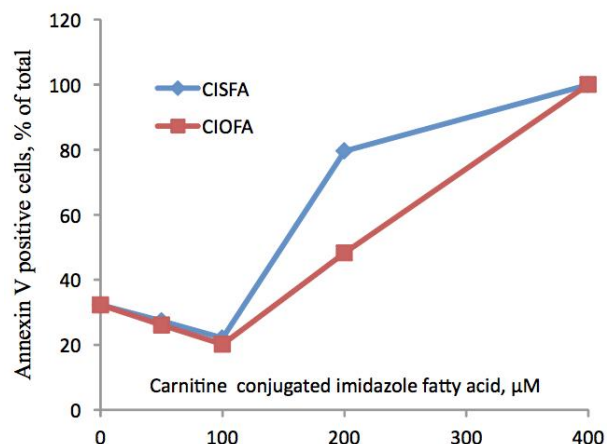


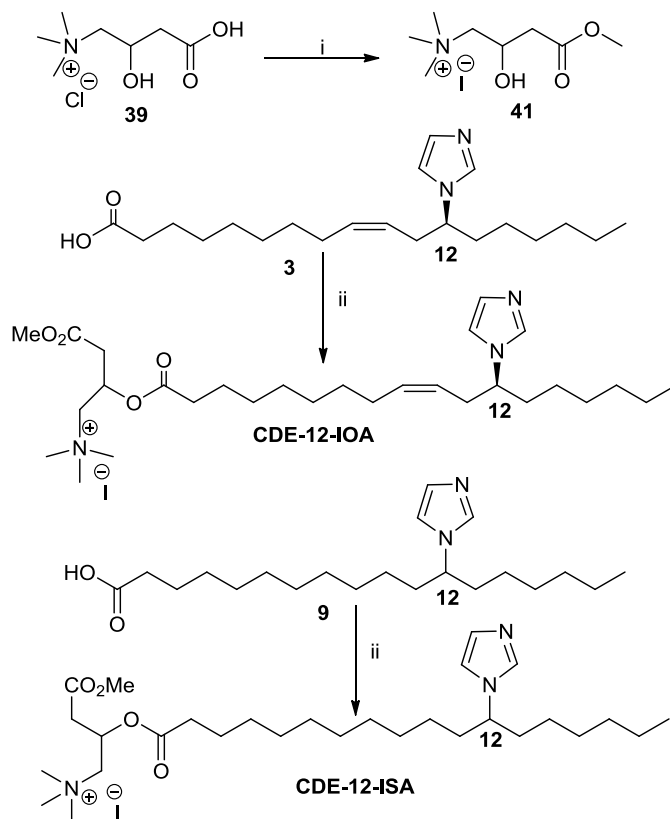
Figure 20: Radiomitigative effects of carnitine conjugated imidazole fatty acids in mouse embryonic cells. Cells were seeded in 6-well plate at a density of $0.05 \times 10^6/\text{well}$ and allowed to attach overnight. Fatty acid conjugates (final, 50, 100, 200 and 400 μM) were added to cells 15 min after gamma-irradiation exposure (10 Gy). Irradiation induced cell death was assessed by Annexin V/Propidium iodide staining assay using flow cytometry after 48 hours incubation. Data presented are the Annexin V positive cells.

Figure 20 shows that the carnitine esters 12-CIOFA and 12-CISFA have mild radiomitigative effects up to 100 μM concentration. At concentrations of 12-CIOFA and 12-CISFA higher than 100 μM , cytotoxic properties were shown to dominate.

We inferred that the problem can be caused by either the detergent like properties of the compounds or cleavage of carnitine ester before inhibition. Detrimental effects of free fatty acids on mitochondria result in elevated levels of ROS via uncoupling of oxidative phosphorylation which further reduces the intracellular levels of glutathione by deterioration of endogenous antioxidant defence mechanism. Incorporation of another methyl ester group on carnitine such as, methyl carnitine ester might render hydrophobic and detergent like properties which can

assist in easy absorption into the cells with low cytotoxicity. We hypothesize that the nonspecific esterases may cleave the 3-carboxy ester bond thereby releasing the fatty acid carnitine ester which is later absorbed into the mitochondrion where the carnitine ester is cleaved. Synthesis of double carnitine esters, such as **CDE-12-IOA** and **CDE-12-ISA** are shown **Scheme 9**.

2.6 Synthesis of 2-(((*S,Z*)-12-(1*H*-imidazol-1-yl)octadec-9-enoyl)oxy)-4-methoxy-*N,N,N*-trimethyl-4-oxobutan-1-aminium iodide (CDE-12-IOA) and 2-(((12-(1*H*-imidazol-1-yl)octadecanoyl)oxy)-4-methoxy-*N,N,N*-trimethyl-4-oxobutan-1-aminium iodide (CDE-12-ISA)



Reaction Conditions: (i) Carnitine hydrochloride, 57% HI in water, methanol, reflux, 6h, 60%, (ii) compound **3** or **9**, compound **41**, DCC, DMAP, THF and DCM, 16h.

Scheme 9: Synthesis of **CDE-12-IOA** and **CDE-12-ISA**.

Carnitine methyl ester **41** was prepared by refluxing carnitine hydrochloride **39** in methanol and hydroiodic acid for 16 h.⁷¹ The carnitine methyl ester **41** was esterified with compounds **3** and **9** to give the compound **CDE-12-IOA** and **CDE-12-ISA** respectively (**Scheme 9**).⁷²

3. Conclusion and Future Work

We synthesized six positional isomers of imidazole substituted fatty acids (6, 8, 10, 12, 13, and 14 stearates, plus 12-imidazole substituted oleate) as the TPP ester derivatives. We also prepared the 12-imidazole carnitine single esters and double esters as the saturated and unsaturated fatty acid derivatives. TPP-12-IFA and TPP-12-ISA derivatives were tested in *in vitro* and *in vivo*, and showed dose dependent inhibition of cyt c peroxidase activity. The TPP imidazole fatty acid derivatives and the single carnitine esters were tested only *in vitro*. Of all these tested compounds, compound TPP-8-ISA has more inhibitory activity *in vitro* against the cyt c peroxidase activity compared to the other compounds. Single carnitine esters showed low inhibitor activity against the peroxidase activity. We are hoping to receive biological data on the double carnitine esters in the near future. After getting those results we will confirm whether it is worth pursuing the carnitine double esters.

TPP-8-ISA was tested as a racemic mixture *in vitro* against the cyt c peroxidase activity. Both isomers of the TPP-8-ISA will be synthesized separately and will be tested against the cyt c peroxidase activity in order to affirm which isomer shows high inhibition. 8-hydroxy fatty acid will be used as a starting material to synthesize the two isomers of TPP-8-ISA. In the beginning, secondary alcohol will be oxidised to a ketone followed by converting it back to an alcohol in the presence of chiral catalyst to obtain a single isomer of an 8-hydroxy fatty acid. This single isomer (R or S) of the alcohol will then be converted into another isomer (S or R) by using Mitsunobu reaction. These two alcohols will then be transformed into two single isomers (R and S) of TPP-8-ISA.

4. Experimental

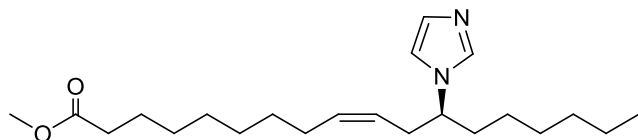
General

All reagents were purchased from Sigma – Aldrich Co., Oakville, Ontario. All glassware was dried in an oven at 90°C overnight, and then cooled in desiccators before use. Cooling baths were prepared with dry ice in ethyl acetate for -78°C and ice/salt mixture for 0°C – 15°C. All air and moisture sensitive reactions were performed under nitrogen gas. THF and Et₂O were distilled with sodium/benzophenone before use. DCM was dried over CaH₂. Methanol was distilled from Mg/iodine. Reagent grade solvents were used for extraction and column purifications. Distilled water was used for all aqueous workups.

Analytical thin layer chromatography (TLC) was done on Merck 0.25 mm pre-coated silica gel 60 A° F-254 on aluminum sheets and visualized under UV light or stained with iodine in silica gel, KMnO₄ solution, ninhydrin solution or vanilin solution. Column chromatography was performed on silica gel 230 – 400 mesh purchased from SiliCycle Inc, Quebec, Canada.

¹H NMR and ¹³C NMR spectra were measured on a Bruker Advance DPX – 300 digital FT – NMR spectrometer (300 and 75 MHz respectively) and Bruker 600 NMR spectrometer (600 and 151 MHz respectively) in CDCl₃ with residual chloroform as internal reference (7.281 ppm for ¹H and 77.0 for ¹³C) unless otherwise mentioned. Chemical shifts were reported as δ values and coupling constants (*J*) were reported in Hertz (Hz). The following abbreviations were used as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). Mass spectra (MS) were recorded on Carlo Erba/Kratos GC/MS Concept 1S double focusing mass spectrometer interfaced to a Kratos DART acquisition system and a Sun SPARC workstation. Samples were introduced through a direct inlet system. Ions were generated by using electron impact (EI) or Fast Atom Bombardment (FAB) and were reported as *m/z* values.

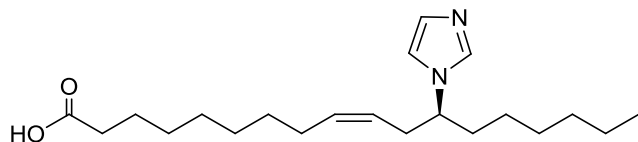
Synthesis of (*R,Z*)-methyl 12-(1*H*-imidazol-1-yl)octadec-9-enoate (**2**)



Methyl ricinoleate (**1**) (10.0 g, 32 mmol), TEA (5.8 ml, 41.60 mmol) and DMAP (0.39 g, 3.2 mmol) were dissolved in dry DCM (150 ml), cooled to 0°C, then methanesulfonyl chloride (2.72 ml, 33.60 mmol) was added slowly under nitrogen gas, stirred at room for another 2 h. The reaction mixture was diluted with DCM, washed with water, brine solution, dried over anhydrous MgSO₄ and then concentrated. This crude compound was heated with imidazole without solvent at 70°C for 16 h under a nitrogen atmosphere. The crude product was purified by silica gel column chromatography (2% MeOH in DCM) and provided compound **2** (3.95 g, 34%) as a gummy liquid.

TLC	R _f = 0.45 (5% MeOH in DCM, KMnO ₄ and iodine active.)
¹ H NMR	(300 MHz, CDCl ₃) δ: 7.46 (s, 1H), 7.07 (s, 1H), 6.91 (s, 1H), 5.46 – 5.40 (m, 1H), 5.20 - 5.17 (m, 1H), 3.95 - 3.90 (m, 1H), 3.68 (s, 3H), 2.51 – 2.42 (m, 2H), 2.31(t, <i>J</i> = 7.5 Hz, 2H), 1.93 – 1.91 (m, 2H), 1.82 – 1.72 (m, 3H), 1.65 – 1.58 (m, 2H), 1.28 – 1.56 (m, 16H), 0.90 – 0.84 (m, 3H).
¹³ C NMR	(151 MHz, CDCl ₃) δ: 174.28, 135.46, 134.97, 122.35, 121.17, 118.67, 61.80, 54.80, 51.44, 34.62, 34.00, 33.40, 31.43, 29.20, 29.03, 28.96, 28.72, 27.28, 25.81, 24.84, 22.45, 13.96.
MS (EI)	m/z: [M ⁺] 362 (6.9%), 165 (100%), 69 (21%), 55 (16.8%).
HRMS	Calculated: 362.5493 Found: 362.2926

Synthesis of (*S*, *Z*)-12-(1*H*-imidazol-1-yl)octadec-9-enoic acid (**3**)



To a stirred solution of compound **2** (2.50 g, 6.9 mmol) in a mixture of MeOH (30 ml) and water (10 ml), was added NaOH (0.83 g, 20.67 mmol) at 0°C, which was then allowed to warm to room temperature and stirring was continued for another 3 h. The reaction mixture was concentrated to remove methanol, then the aqueous layer was acidified with 1N HCl to pH 4, and extracted with DCM (3x50 ml). The combined organic layers were washed with brine solution, dried over anhydrous MgSO₄ and then concentrated. The crude product was purified by silica gel column chromatography (5% MeOH in DCM) to obtain compound **3** (2.16 g, 89%) as a pale yellow gummy liquid.

TLC R_f = 0.54 (10% MeOH in DCM, iodine and KMnO₄ active.)

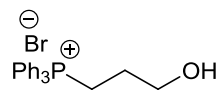
¹H NMR (300 MHz, CDCl₃) δ : 10.06 (b, 2H), 9.19(s, 1H), 7.47(s, 1H), 7.21(s, 1H), 5.52 – 5.40 (m, 1H), 5.24 – 5.16 (m, 1H), 4.45 – 4.35(m, 1H), 2.60 – 2.53(m, 2H), 2.34 – 2.29 (m, 2H), 1.87 (m, 4H), 1.61 – 1.54 (m, 2H), 1.26 – 1.11 (b, 17H), 0.86 – 0.82 (m, 3H).

¹³C NMR (75 MHz, CDCl₃) δ : 177.41, 135.15, 134.58, 122.25, 120.43, 118.62, 61.80, 34.66, 34.02, 33.42, 31.41, 28.88, 28.66, 28.62, 27.09, 25.80, 24.59, 22.44, 13.96.

MS (EI) m/z : 149 (4.8%), 101 (18.6%), 59 (52.5%), 58 (22.9%), 43 (100%).

HRMS Calculated: 348.5227 Found: 348.2784

Synthesis of (3-hydroxypropyl)triphenylphosphonium bromide (**5**)



3-Bromo-1-propanol **4** (5.0 g, 35.97 mmol) and triphenylphosphine (10.38 g, 39.57 mmol) were dissolved in toluene (100 ml) and heated at 110°C for 16 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The crude compound was washed with 1:4 of DCM:hexane to remove unreacted compound **4** and triphenylphosphine. Compound **5** was so obtained as an off – white solid (13.06 g, 95%).

TLC R_f = 0.25 (10% MeOH in DCM, iodine and KMnO₄ active.)

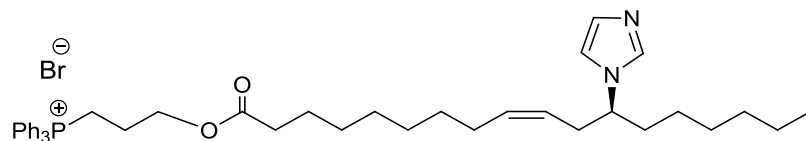
¹H NMR (600 MHz, CDCl₃) δ : 7.80 – 7.75 (m, 9H), 7.71 – 7.68 (m, 6H), 3.82 (m, 2H), 3.76 – 3.72 (m, 2H), 3.25 (br, 1H), 1.87 – 1.81 (m, 2H).

¹³C NMR (151 MHz, CDCl₃) δ : 135.12, 133.55, 133.48, 130.62, 130.54, 118.64, 118.06, 60.40, 60.29, 25.84, 20.32, 19.98.

MS (EI) m/z : 149 (4.8%), 101 (18.6%), 59 (52.5%), 58 (22.9%), 43 (100%).

HRMS Calculated: 348.5227 Found: 348.2784

Synthesis of (*S,Z*)-(3-((12-(1*H*-imidazol-1-yl)octadec-9-enoyl)oxy)propyl)triphenylphosphonium bromide (TPP-12-IOA)



Compound **3** (0.54 g, 1.55 mmol) and compound **5** (0.622 g, 1.55 mmol) were dissolved in DCM (10 ml), cooled to 0°C, to which was then added DCC (0.32g, 1.55mmol) under nitrogen atmosphere. The reaction solution was stirred overnight at room temperature, and the precipitated DCU removed by filtration. The filtrate was concentrated by evaporation. The crude product was purified by silica gel column chromatography (4% MeOH in DCM) to give title compound **TPP-12-IOA** (0.96mg, 85%) as gummy liquid.

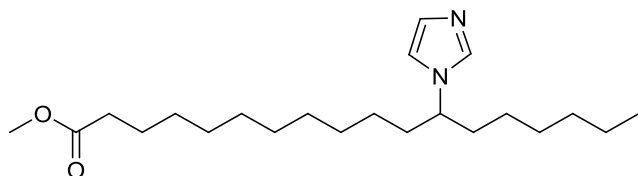
TLC R_f = 0.45 (10% MeOH in DCM, KMnO₄ and iodine active).

¹H NMR (600 MHz, CDCl₃) δ : 7.85 – 7.77 (m, 9H), 7.70 – 7.67 (m, 6H), 7.44 (s, 1H), 7.01 (s, 1H), 6.86 (s, 1H), 5.37 (m, 1H), 5.37 (m, 1H), 4.34 (t, J = 6 Hz, 2H), 3.95 – 3.91 (m, 3H), 2.4 (bs, 1H), 2.23 – 2.20 (m, 2H), 1.99 – 1.98 (m, 2H), 1.85 – 1.84 (m, 2H), 1.52 – 1.49 (m, 2H), 1.25 – 1.15 (m, 16H), 0.82 – 0.80 (m, 3H).

¹³C NMR (151 MHz, CDCl₃) δ : 173.41, 135.19, 133.72, 133.65, 133.40, 130.61, 130.53, 123.97, 118.24, 117.67, 63.14, 63.02, 58.74, 35.44, 34.23, 34.09, 31.54, 29.30, 29.10, 29.01, 28.81, 27.21, 25.98, 24.76, 22.48, 22.25, 19.91, 19.56, 14.01.

MS (EI) m/z : [M+1] 652.5 (46.5%), 651.5 (100%), 326.8 (11.4%), 326.3 (27.8%).

Synthesis of methyl 12-(1*H*-imidazol-1-yl)octadecanoate (**7**)



Compound **6** (2.0 g, 6.36 mmol), TEA (1.15 ml, 8.27 mmol) DMAP (78 mg, 0.64 mmol) were dissolved in DCM (30 ml) under nitrogen atmosphere, cooled 0°C, then methanesulfonyl

chloride (0.54 ml, 6.68 mmol) was added drop wise for 10 minutes. The reaction was stirred at room temperature for another 2 h. The reaction was diluted with DCM (40 ml), washed with water (40 ml), 1N HCl (20 ml), brine solution and dried over anhydrous MgSO_4 and concentrated. The crude mesylated compound and imidazole (0.87 g, 12.73 mmol) mixture was heated at 70°C for 16 hours. The crude compound was purified by using silica gel column (2% MeOH in DCM) to give compound **8** as light yellow liquid (0.81 g, 35%).

TLC R_f = 0.55 (5% MeOH in DCM, KMnO_4 and iodine active).

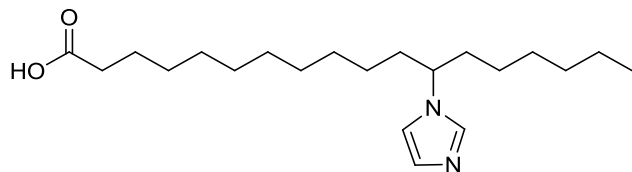
^1H NMR (300 MHz, CDCl_3) δ : 7.35 (s, 1H), 6.95 (s, 1H), 6.78 (s, 1H), 3.78 (m, 1H), 3.54 (s, 3H), 2.18 (t, J = 7.5 Hz, 2H), 1.61 – 1.47 (m, 6H), 1.15 – 0.94 (m, 22H), 0.76 – 0.71 (m, 3H).

^{13}C NMR (75 MHz, CDCl_3) δ : 174.04, 136.36, 129.26, 116.25, 58.53, 51.23, 36.43, 36.20, 34.56, 34.05, 33.92, 31.63, 31.46, 29.26, 29.21, 29.06, 28.97, 28.74, 25.93, 25.90, 25.16, 24.79, 22.38.

MS (EI) m/z : [M^+] 364 (29.5%), 363 (29.2%), 223 (63.8%), 221 (100%), 171 (32.6%), 95 (30.6%), 82 (33.7%), 69 (77.8%), 55 (50.6%)

HRMS Calculated: 364.5652 Found: 364.3085.

Synthesis of 12-(1*H*-imidazol-1-yl)octadecanoic acid (**8**)



NaOH (263 mg, 6.58 mmol) was added to a stirred solution of compound **7** (800 mg, 2.19 mmol) in methanol (9 ml) and water (3 ml) mixture at 0°C. Reaction was stirred at room temperature for 2h, then the reaction mixture was concentrated to remove methanol. Aqueous layer was acidified with 1N HCl up to pH 4, extracted with DCM (3x40 ml), combined organic layers were washed with brine solution, dried over MgSO₄ and concentrated. Compound was purified by silica gel column (4% MeOH in DCM) to give compound **8** as gummy liquid (682 mg, 89%)

TLC R_f = 0.52 (10% MeOH in DCM, KMnO₄ and iodine active).

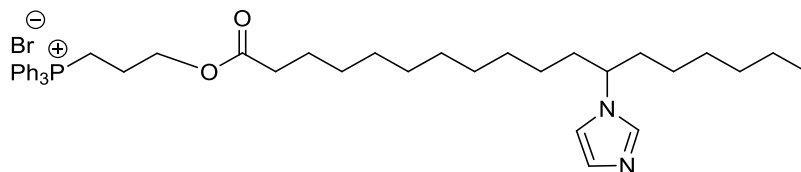
¹H NMR (300 MHz, CDCl₃) δ : 10.899 (s, 2H), 8.68 (s, 1H), 7.33 (s, 1H), 7.05 (s, 1H), 4.17 (m, 1H), 2.29 (t, J = 7.5 Hz, 2H), 1.83 – 1.54 (m, 6H), 1.52 – 1.19 (m, 20H), 1.03 – 1.02 (br, 2H), 0.84 – 0.80 (m, 3H).

¹³C NMR (75 MHz, CDCl₃) δ : 177.76, 135.19, 123.11, 117.62, 61.04, 35.76, 35.70, 34.46, 31.44, 29.07, 29.01, 28.91, 28.71, 25.81, 25.71, 24.87, 22.44, 13.94.

MS (EI) m/z : [M⁺] 350 (2%), 265 (45.5%), 165 (78.7%), 95 (19.2%), 69 (100%), 55 (38.8%), 43 (37.7%), 41 (33%).

HRMS Calculated: 350.5386 Found: 350.2918.

Synthesis of (3-((12-(1*H*-imidazol-1-yl)octadecanoyl)oxy)propyl)triphenylphosphonium bromide (TPP-12-ISA)



DCC (359 mg, 1.74 mmol) was added to a stirred solution of compound **8** (610 mg, 1.74 mmol) and compound **5** (698 mg, 1.74 mmol) in DCM (10 ml) at 0°C under N₂ gas. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was filtered, and concentrated. The crude compound was purified by silica gel column (5% MeOH in DCM) to give compound **TPP-12-ISA** (1.05 g, 82%) as gummy liquid.

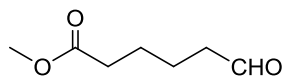
TLC R_f = 0.35 (10% MeOH in DCM, KMnO₄ active).

¹H NMR (300 MHz, CDCl₃) δ : 7.80 – 7.67 (m, 15H), 7.50 (s, 1H), 7.08 (s, 1H), 6.90 (s, 1H), 4.39 (t, J = 6.3 Hz, 2H), 4.12 – 4.02 (m, 2H), 3.93 – 3.85 (m, 1H), 2.24 (t, J = 7.5 Hz, 2H), 2.08 – 1.95 (m, 2H), 1.78 – 1.67 (m, 4H), 1.56 – 1.51 (m, 2H), 1.26 – 1.06 (m, 23H), 0.87 – 0.83 (m, 3H).

¹³C NMR (CDCl₃) δ 173.1, 134.9, 134.9, 133.4, 133.3, 130.4, 130.2, 118.2, 117.0, 116.2, 62.9, 62.6, 58.4, 36.0, 33.8, 31.2, 29.0, 28.8, 28.7, 28.5, 25.7, 25.6, 24.5, 22.2, 21.9, 19.8, 19.1, 13.7.

MS (FAB) m/z : [M⁺] 653 (100%), 375 (9%), 319 (12%), 275 (12%), 262 (14%), 183 (12%), 69 (55%).

Synthesis of methyl 6-oxohexanoate (**10**)



Cyclohexene **9** (6.16 g, 75.02 mmol) and NaHCO₃ (2.0 g, 24.01 mmol) were suspended in 250 ml of DCM, cooled to -78°C, then ozone gas was bubbled through the solution until a light blue color appear. The ozone outlet was removed from the reaction and nitrogen gas bubbled through

the solution to remove excess ozone, then the solution was allowed to room temperature and filtered off the NaHCO_3 , then 80 ml of benzene was added to the mixture and the volume reduced to 50 ml. The solution was diluted with another 225 ml of dichloromethane, then triethylamine (22 ml, 112.5 mmol) and acetic anhydride (19.63 ml, 225.1 mmol) were added at 0°C . The mixture was warmed to room temperature and stirring continued overnight. The mixture was diluted with 100 ml of DCM, washed with 150 ml of 0.1N HCl, 150 ml of 10% NaOH solution, brine solution, dried on anhydrous MgSO_4 , and concentrated. The crude compound was distilled at 60°C under 0.1 mm vacuum to obtain compound **10** as colorless liquid (6.8 g, 63%).

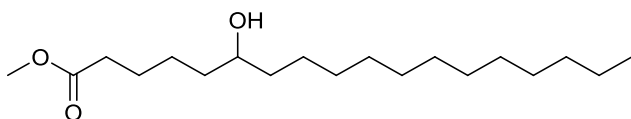
TLC $R_f = 0.5$ (20% ethyl acetate in hexanes, iodine and KMnO_4 stain).

^1H NMR (300 MHz, CDCl_3) δ : 9.76 (t, $J = 1.8$ Hz, 1H), 3.66 (s, 3H), 2.48 – 2.43 (m, 2H), 2.36 – 2.31 (m, 2H), 1.69 – 1.66 (m, 4H).

^{13}C NMR (151 MHz, CDCl_3) δ : 201.85, 173.43, 51.28, 43.26, 33.48, 24.17, 21.32.

MS (EI) m/z : 145 $[\text{M}+1]^+$ (3.0%), 129 (28.3%), 114 (45.9%), 113 (25.4%), 101 (51.5%), 100 (26.6%), 87 (53.4%), 74 (79.9%), 59 (100%), 55 (88.4%), 43 (82.4%), 41 (49.8%).

Synthesis of methyl 6-hydroxyoctadecanoate (**11**)

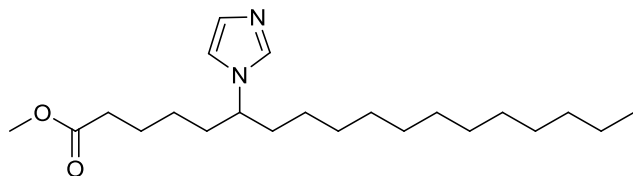


Magnesium metal (0.359 g, 14.56 mmol) was added into dry THF (10 ml) in a 100 ml two-necked round bottom flask which was equipped with condenser and nitrogen gas inlet. The

flask was heated to 70°C, then four to five drops of 1, 2-dibromoethane was added to the reaction mixture, followed by slow addition of 1-bromododecane (2.92 g, 11.726 mmol) at reflux and stirring continued another 30 min. The Grignard reagent was added drop wise to a stirred solution of methyl 6-oxohexanoate (1.31 g, 11.49 mmol) in THF (15 ml) at -20°C. The reaction mixture was slowly brought to room temperature, and stirring continued for another 2h. The mixture was quenched with saturated ammonium chloride solution, extracted with (3x40ml) of ethyl acetate and the combined organic layers were washed with brine solution, dried on anhydrous MgSO₄, concentrated. The crude compound was purified by silica gel column chromatography (2:8 ethyl acetate and hexanes) to offer compound 11 as a white coloured low melting solid (1.55 g, 47%).

TLC	R _f = 0.42 (20% ethyl acetate in hexanes, iodine and KMnO ₄ active).	
¹ H NMR	(300 MHz, CDCl ₃) δ: 3.68 (s, 3H), 3.60 (m, 1H), 2.34 (t, <i>J</i> = 7.5 Hz, 2H), 1.70 – 1.61 (m, 2H), 1.52 – 1.27 (m, 26H), 0.91 – 0.87 (m, 3H).	
¹³ C NMR	(75 MHz, CDCl ₃) δ: 174.16, 71.55, 51.42, 37.49, 36.95, 33.97, 31.88, 29.67, 29.61, 29.31, 25.63, 25.17, 24.87, 22.64, 14.06.	
MS (EI)	m/z: 264 (4.8%), 158 (9.5%) 145 (20.8%), 113 (38.4%), 87 (49%), 67 (41.8%), 55 (99.5%), 41 (100%).	
HRMS	Calculated: 314.5032	Found: 296.2717 – H ₂ O

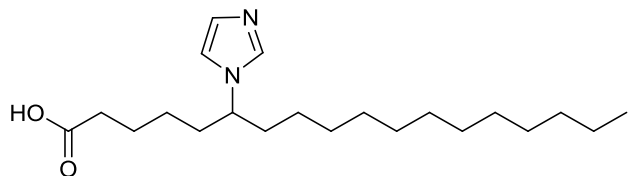
Synthesis of methyl 6-(1*H*-imidazol-1-yl)octadecanoate (**12**)



Methanesulfonyl chloride (0.39 ml, 4.99 mmol) was added drop wise to a stirred solution of compound **11** (1.55 g, 4.94 mmol) and TEA (0.89 ml, 6.42 mmol) in dry DCM (30 ml) at 0°C. The reaction mixture was warmed to room temperature and stirring continued for 2h. The reaction mixture diluted with 50 ml of DCM, the organic layer was washed with water and brine solution, dried on anhydrous MgSO₄, concentrated. This crude compound was combined with imidazole (0.67 g, 9.88 mmol) heated at 80°C for 16 h. The crude compound was purified by silica gel column (2% methanol in DCM) to obtain compound **12** as light brown liquid (605 mg 33.7%).

TLC	R _f = 0.45 (5% methanol in dichloromethane, iodine and KMnO ₄ active).	
¹ H NMR	(300 MHz, CDCl ₃) δ: 7.46 (s, 1H), 7.07 (s, 1H), 6.87 (s, 1H), 3.88 (m, 1H), 3.63 (s, 3H), 2.24 (t, <i>J</i> = 7.5 Hz, 2H), 1.71 - 1.48 (m, 6H), 1.23 - 1.09 (m, 22H), 0.88 – 0.84 (m, 3H).	
¹³ C NMR	(75 MHz, CDCl ₃) δ: 173.75, 58.47, 51.48, 36.27, 35.98, 33.67, 31.87, 29.58, 29.55, 29.47, 29.36, 29.30, 29.16, 26.02, 25.56, 24.40, 22.65, 14.08.	
MS (EI)	m/z: 364 (8.6%), 363 (10.7%), 249 (32.2%), 95 (24.3%), 82 (15.3%), 81(16.0%) 69 (100%), 57 (20.2%) 55(41.7%), 43 (81.9%).	
HRMS	Calculated: 364.5652	Found: 364.3092.

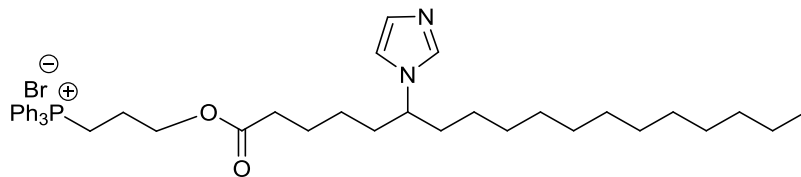
Synthesis of 6-(1*H*-imidazol-1-yl)octadecanoic acid (**13**)



Sodium hydroxide (0.11 g, 2.75 mmol) was added to a stirred solution of compound **12** (0.5 g, 1.37 mmol) in a mixture of methanol (9 ml) and water (3 ml) at 0°C, and stirring continued for another 3 hours at room temperature. The reaction mixture was concentrated to remove the methanol. The aqueous layer was diluted with water and acidified with 1N HCl up to pH 4, extracted with dichloromethane (3 x 15 ml), and the combined organic layers were washed with brine solution, dried on anhydrous MgSO₄, and concentrated. The crude product was purified on silica gel column (5% MeOH in DCM) to obtain compound **13** as gummy liquid (370 mg, 77%).

TLC	R _f = 0.52 (10% MeOH in DCM, KMnO ₄ and iodine active).	
¹ H NMR	(300 MHz, CDCl ₃) δ: 12.95 (s, 1H), 7.80 (s, 1H), 7.13 (s, 1H), 6.92 (s, 1H), 4.00 – 3.98 (m, 1H), 2.37 – 2.20 (m, 2H), 1.80 - 1.51 (m, 6H) 1.30 – 1.08 (m, 22H), 0.90 – 0.86 (m, 3H).	
¹³ C NMR	(75 MHz, CDCl ₃) δ: 176.98, 135.96, 126.87, 116.68, 59.27, 36.17, 35.79, 34.47, 31.89, 29.61, 29.56, 29.49, 29.35, 29.32, 29.16, 26.03, 25.35, 24.48, 22.67, 14.10.	
MS (EI)	m/z: 351 (2.5%), 350 (10.5%), 349 (10.2%), 249 (46.3%), 181 (14.2%), 95 (23.3%), 69 (100%), 55 (38%), 43 (58%), 41(63%).	
HRMS	Calculated: 350.5386	Found: 350.2935.

Synthesis of (3-((6-(1*H*-imidazol-1-yl)octadecanoyl)oxy)propyl)triphenylphosphonium bromide (TPP-6-ISA)



Compound **13** (300 mg, 0.857 mmol) and compound **5** (344 mg, 0.858 mmol) were dissolved in dry DCM (10 ml), then DCC (177 mg, 0.858 mmol) was added at 0°C. The reaction mixture was stirred for 16h at room temperature. The mixture was filtered and concentrated, purified by silica gel column chromatography (6% MeOH in DCM) to obtain compound **TPP-6-ISA** as gummy liquid (420 mg, 67%).

TLC R_f = 0.43 (15% MeOH in DCM, iodine and KMnO₄ active).

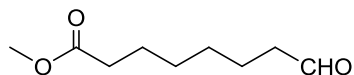
¹H NMR (600 MHz, CDCl₃) δ : 7.74 - 7.60 (m, 15H), 7.38 (s, 1H), 6.89 (s, 1H), 6.80 (s, 1H), 4.20 (s, 2H), 3.80 – 3.79 (m, 3H), 2.11 – 2.07 (m, 2H), 1.87 – 1.86 (m, 2H), 1.59 – 1.58 (m, 4H), 1.46 - 1.35 (m, 2H), 1.11 – 0.93 (m, 24H), 0.74 – 0.72 (m, 3H).

¹³C NMR (151 MHz, CDCl₃) δ : 172.84, 136.29, 135.16, 135.15, 133.62, 133.56, 130.57, 130.48, 128.96, 118.06, 117.49, 116.50, 63.15, 63.03, 58.47, 36.16, 35.85, 33.68, 31.77, 29.48, 29.45, 29.38, 29.28, 29.19, 29.08, 25.93, 25.42, 24.17, 22.55, 22.10, 19.89, 19.54, 14.03.

MS (FAB) m/z : [M+1]⁺ 654 (46.9%), [M]⁺ 653 (100%), 319 (12.1%), 303(10.5%), 275 (11%), 262 (12.7%), 183(11.5%), 69 (30.8%).

HRMS Calculated: 653.8953 Found: 653.4220.

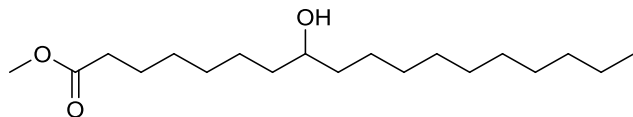
Synthesis of methyl 8-oxooctanoate (**15**)



Cis-cyclooctene **14** (10.0 g, 90.74 mmol) and NaHCO₃ (2.44 g, 29.05 mmol) were suspended in DCM (400 ml), and then ozone gas was bubbled through the solution at -78°C until a light blue color appeared. The ozone outlet was removed from the mixture and nitrogen gas bubbled through the solution to remove excess ozone. It was allowed to warm to room temperature and filtered. 100 ml of benzene was added to the mixture and the volume reduced by evaporation to 100 ml. The solution was diluted with another 400 ml of DCM, and then triethylamine (18.94 ml, 136.1 mmol) and acetic anhydride (25.7 ml, 272.2 mmol) were added to it at 0°C and stirring continued overnight at room temperature. Mixture was diluted with 200 ml of DCM, washed with 200 ml of 0.1N HCl, 200 ml of 10% NaOH solution, brine solution, dried on anhydrous MgSO₄, and concentrated. The crude compound was distilled at 96°C under 0.1 mm vacuum to obtain compound **15** as colorless liquid (10.9g, 70%).

TLC	R _f = 0.45 (20% Ethyl acetate in hexanes, iodine and KMnO ₄ stain).	
¹ H NMR	(300 MHz, CDCl ₃) δ: 9.52 (t, <i>J</i> = 1.8 Hz, 1H), 3.42 – 3.40 (m, 3H), 2.21 - 2.17 (m, 2H), 2.08 - 2.03 (m, 2H), 1.40 – 1.39 (m, 4H), 1.12 – 1.11(m, 4H).	
¹³ C NMR	(75 MHz, CDCl ₃) δ: 202.04, 173.56, 51.00, 43.41, 33.53, 28.54, 28.48, 24.40, 21.55.	
MS (EI)	m/z: 173 [M+1] (2.4%), 171 (5.4%), 157 (24.35%), 138 (49.7%), 129 (24.6%), 97 (32.9%), 87 (37.3%), 74 (100%), 69 (50.7%).	
HRMS	Calculated: 172.2215	Found: 171.1019.

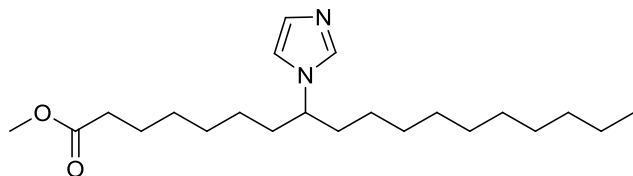
Synthesis of methyl 8-hydroxyoctadecanoate (**16**)



Magnesium metal (0.209 g, 8.71 mmol) was added in dry THF (10 ml) in a 50 ml two-necked round bottom flask fitted with a condenser and nitrogen gas inlet. 1-Bromodecane (1.3 g, 5.81 mmol) was added slowly at reflux temperature after initiating the reaction with 1,2-dibromoethane and stirring continued for 30 min. The cooled Grignard reagent was added drop wise to a stirred solution of compound **15** (1.0 g, 5.8 mmol) in THF (10 ml) at -20°C , and the reaction mixture was slowly warmed to room temperature and stirring continued for 2h. The reaction mixture was quenched with saturated ammonium chloride solution, extracted with (3 x 25 ml) ethyl acetate, and the combined organic layers were washed with brine solution, dried on anhydrous MgSO_4 , concentrated. The crude was purified on silica gel column chromatography (20% EtOAc and hexanes) to obtain compound **16** as off-white solid (450 mg, 47%).

TLC	$R_f = 0.4$ (20% ethyl acetate in hexanes, iodine and KMnO_4 stain).	
^1H NMR	(300 MHz, CDCl_3) δ : 3.66 (s, 3H), 3.57 (m, 1H), 2.30 (t, $J = 7.5$ Hz, 2H), 2.17 (s, 1H), 1.62 – 1.58 (m, 4H), 1.42 - 1.25 (m, 26H), 0.89 - 0.85 (m, 3H).	
^{13}C NMR	(75 MHz, CDCl_3) δ 174.28, 71.94, 51.44, 37.49, 37.35, 34.05, 31.89, 29.70, 29.61, 29.32, 29.29, 29.09, 25.64, 25.43, 24.86, 22.67, 14.10.	
MS (EI)	m/z : 296 $[\text{M}-18]^+$ (0.5%), 173 (43.4%), 144 (53.3%), 141 (100%).	
HRMS	Calculated: 314.5032	Found: 296.2718 ($-\text{H}_2\text{O}$).

Synthesis of methyl 8-(1*H*-imidazol-1-yl)octadecanoate (**17**)



To a stirred solution of compound **16** (0.92 g, 2.93 mmol) DMAP (30 mg) and TEA (0.42 ml, 6.42 mmol) in dry DCM (30 ml) at 0°C, then methanesulfonyl chloride (0.23 ml, 2.96 mmol) was added under N₂. The reaction mixture was stirred at room temperature for 2h. The reaction was diluted with DCM (30 ml), the organic layer was washed with water and brine solution, dried on anhydrous MgSO₄, concentrated and dried under vacuum. The crude compound was mixed with imidazole (0.45 g, 5.86 mmol) and then heated at 70°C for 16 h. The crude compound was purified on silica gel chromatography (2% MeOH in DCM) to obtain compound **17** as light brown liquid (370 mg, 35%).

TLC R_f = 0.4 (5% MeOH in DCM, Iodine and KMnO₄ stain).

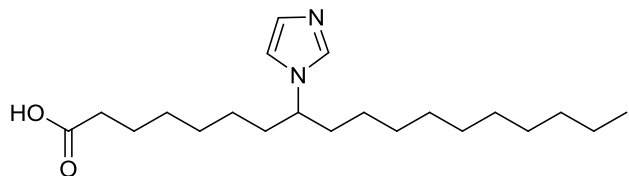
¹H NMR (300 MHz, CDCl₃) δ : 7.46 (s, 1H), 7.07 (s, 1H), 6.88 (s, 1H), 3.91 - 3.82 (m, 1H), 3.65 (s, 3H), 2.31 - 2.24 (m, 2H), 1.77 - 1.54(m, 6H), 1.23 - 1.09 (m, 22H), 0.91 - 0.86 (m, 3H).

¹³C NMR (75 MHz, CDCl₃) δ : 174.09, 129.41, 116.45, 58.70, 51.43, 36.30, 36.23, 33.91, 31.85, 29.51, 29.48, 29.36, 29.25, 29.19, 28.83, 26.04, 25.88, 24.73, 22.63, 14.07.

MS (EI) m/z : 364 (14.8%), 363 (21.1%), 333 (10.8%), 224(12.1%), 223 (63%), 222 (19.5%), 221(100%), 69 (85.5%), 55(21.1%).

HRMS Calculated: 364.5652 Found: 364.3088.

Synthesis of 8-(1*H*-imidazol-1-yl)octadecanoic acid (**18**)



Sodium hydroxide (0.67 g, 1.68 mmol) was added to a stirred solution of compound **17** (0.305 g, 0.84 mmol) in a mixture of methanol (9 ml) and water (3 ml) at 0°C, and stirring continued another 3h at room temperature. Methanol was removed from mixture by evaporation. The pH of reaction mixture was adjusted to 4 with 1N HCl and extracted with DCM (3 x 15 ml). The combined organic layers were washed with brine solution, dried on anhydrous MgSO₄, and concentrated. The crude compound was purified by silica gel column chromatography (5% MeOH in DCM) to obtain compound **18** as gummy liquid (370mg, 83%).

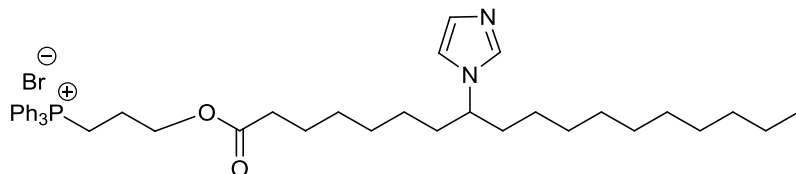
¹H NMR (300 MHz, CDCl₃) δ: 8.73 - 8.68 (m, 3H), 7.33 (s, 1H), 7.05 (s, 1H) 4.19 – 4.15 (m, 1H), 2.32 – 2.27 (m, 2H), 1.80 - 1.54 (m, 6H) 1.33 - 1.06 (m, 22H), 0.88 – 0.84 (m, 3H).

¹³C NMR (75 MHz, CDCl₃) δ: 131.93, 130.90, 130.78, 128.83, 128.68, 59.36, 36.20, 36.14, 31.86, 29.52, 29.49, 29.36, 29.26, 29.17, 28.91, 28.87, 26.04, 25.89, 24.91, 22.66, 14.10.

MS (EI) *m/z* 351 (2.8%), 350 (8.0%), 230 (14.7%), 222 (20.9%), 221 (100%), 216 (37.8%), 215 (67.7%), 209 (60.4%), 202 (65.4%), 201 (41.1%).

HRMS Calculated: 350.5386 Found: 350.2936.

Synthesis of (3-((8-(1*H*-imidazol-1-yl)octadecanoyl)oxy)propyl)triphenylphosphonium bromide (TPP-10-ISA)



Compound **18** (225 mg, 0.643 mmol) and compound **5** (258 mg, 0.643 mmol) were dissolved in dry DCM then added DCC (146 mg, 0.708 mmol) at 0°C. The reaction mixture was stirred at room temperature for another 16h. The mixture was filtered and concentrated to remove the formed DCU. The crude compound was purified by silica gel column chromatography (5.5% MeOH in DCM) to obtain a gummy liquid (330 mg, 70%).

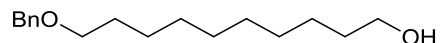
TLC R_f = 0.43 (15% MeOH in DCM, iodine and KMnO₄ active).

¹H NMR (600 MHz, CDCl₃) δ : 7.79-7.63 (m, 15H), 7.45 (s, 1H), 6.97 (s, 1H), 6.85 (s, 1H), 4.27(m, 2H), 3.86 – 3.81 (m, 3H), 2.16 – 2.14 (m, 2H), 1.95 – 1.92 (m, 2H), 1.66 – 1.60 (m, 4H), 1.43 – 1.41 (m, 2H), 1.21-1.13 (m, 22H), 0.99 - 0.97 (m, 2H), 0.80 – 0.77 (m, 3H).

¹³C NMR (151 MHz, CDCl₃) δ : 173.23, 136.28, 135.21, 135.19, 135.11, 133.66, 133.59, 133.47, 133.41, 130.61, 130.52, 130.44, 130.40, 128.85, 118.12, 117.55, 116.56, 63.12, 63.00, 58.80, 36.23, 36.14, 33.90, 31.83, 31.79, 29.55, 29.51, 29.46, 29.43, 29.33, 29.26, 29.20, 29.14, 28.76, 28.75, 26.00, 25.82, 24.53, 22.59, 22.20, 19.93, 19.57, 14.07.

MS (FAB) m/z : 654 [M+1] (43.4%), 653 (M⁺, 91.9%), 603 (10.3%), 375 (12.8%), 319 (18.3%), 303(16.2%), 289 (13.1%), 275 (18.1%), 183(20.3%), 69 (100%).

Synthesis of 10-(benzyloxy)decan-1-ol (**20**)



A stirred suspension of decane-1, 10-diol **19** (3.0 g, 17.21 mmol), benzyl bromide (2.25 ml, 18.94 mmol) and Ag₂O (5.98g, 25.82 mmol) in dry DCM (50 ml) was refluxed overnight. The reaction mixture filtered to remove solid Ag₂O and filtrate was concentrated. The crude product was purified by silica gel column chromatography (10% EtOAc in Hexane) to afford compound **20** as gummy colorless liquid (2.96 g, 65%).

TLC R_f = 0.55 (20% EtOAc in Hexane, KMnO₄ active).

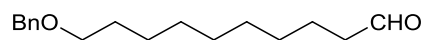
¹H NMR (300 MHz, CDCl₃) δ : 7.37 – 7.28 (m, 5H), 4.52 (s, 2H), 3.60 (t, J = 6.9 Hz, 2H), 3.49 (t, J = 6.6 Hz, 2H), 2.37 (s, 1H), 1.69 – 1.51 (m, 4H), 1.37 – 1.32 (m, 12H).

¹³C NMR (75 MHz, CDCl₃) δ : 138.65, 128.42, 128.35, 127.65, 127.49, 126.89, 72.85, 70.52, 62.78, 32.77, 29.76, 29.58, 29.55, 29.47, 26.20, 25.80.

MS (EI) m/z : 264[M]⁺ (6.5%), 108 (24.9%), 107 (65.6%), 92 (37.8%), 91 (100%) .

HRMS Calculated: 264.2089 Found: 264.2095

Synthesis of 10-(benzyloxy)decanal (**21**)



Compound **20** (8.1g, 30.64 mmol) was dissolved in dry DCM (150 ml), and then PCC (7.86g, 45.95 mmol) was added at 0°C. The reaction mixture was stirred at ambient temperature for 2h.

The reaction mixture was concentrated and purified by silica gel column chromatography (10% EtOAc in Hexane) to give compound **21** as colorless liquid (6.27g, 78%).

TLC R_f = 0.68 (20% EtOAc in Hexane, KMnO_4 active).

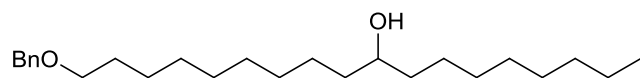
^1H NMR (300 MHz, CDCl_3) δ : 9.76 (t, J = 1.8 Hz, 1H), 7.39 – 7.25 (m, 5H), 4.51 (s, 2H), 3.48(t, J = 6.6 Hz, 2H), 2.44 – 2.39 (m, 2H), 1.67-1.58 (m, 4H), 1.37-1.31 (m, 10H).

^{13}C NMR (75 MHz, CDCl_3) δ : 202.83, 138.72, 128.32, 127.59, 127.45, 72.85, 70.47, 43.88, 29.75, 29.38, 29.35, 29.28, 29.13, 26.16, 22.06.

MS (EI) m/z : 262 $[\text{M}]^+$ (4.1%), 108 (15.8%), 92 (31.5%), 91 (100%), 55 (11.2%).

HRMS Calculated: 262.1933 Found: 262.1935

Synthesis of 18-(benzyloxy)octadecan-9-ol (**22**)



1-Bromooctane (3.57 ml, 23.48 mmol) in THF (10ml) was added slowly to a stirred suspension of Mg metal (0.65 g, 26.83 mmol) and catalytic amount of iodine in dry THF (20 ml) at 70°C. The reaction mixture was stirred at this temperature for another 30 minutes. The Grignard reagent was added slowly to a stirred solution of compound **21** (4.4 g, 16.77 mmol) in THF (30 ml) at 0°C and stirring continued another 2h at room temperature. The reaction was quenched with ammonium chloride solution, extracted with EtOAc (3 x 50 ml), combined organic layers were washed with brine solution, dried over MgSO_4 and concentrated. The crude product was

purified by silica gel column chromatography (12% EtOAc in Hexane) to give compound **22** as low melting white color solid (3.85g, 61%).

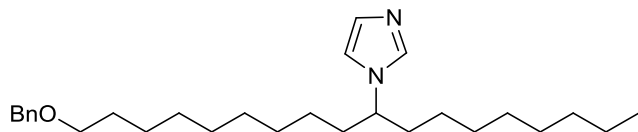
TLC R_f = 0.45 (20% EtOAc in Hexane, KMnO_4 active).

^1H NMR (300 MHz, CDCl_3) δ : 7.37 - 7.28 (m, 5H), 4.52 (s, 2H), 3.60-3.58 (m, 1H), 3.48 (t, J = 6.6 Hz, 2H), 1.68 – 1.59 (m, 2H), 1.52 – 1.30 (m, 28H), 0.92 – 0.88 (m, 3H).

^{13}C NMR (75.46 MHz, CDCl_3) δ 138.72, 128.39, 128.33, 127.62, 127.46, 125.9, 72.85, 71.99, 70.52, 37.5, 31.9, 29.77, 29.74, 29.71, 29.61, 29.58, 29.55, 29.48, 29.30, 26.19, 25.67, 22.68, 14.12.

MS (EI) m/z : 358 $[\text{M}-18]^+$ (2.5%), 107 (25.5%), 92 (19.7%), 91 (100%), 69 (12.8%).

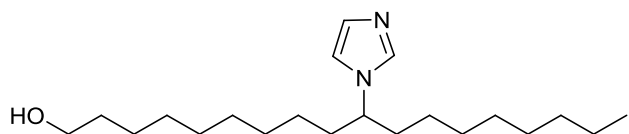
Synthesis of 1-(18-(benzyloxy)octadecan-9-yl)-1H-imidazole (**23**)



Methanesulfonyl chloride (1.64 ml) was added to a stirred solution of compound **22** (6.0 g, 15.93 mmol), DMAP (0.195 g, 1.59 mmol) and TEA (2.9 ml, 20.71 mmol) in dry DCM (90 ml) at 0°C . The reaction mixture was stirred at room temperature another 3h. The reaction mixture was diluted with DCM (100 ml), washed with 1N HCl, water, and brine solution, dried over anhydrous MgSO_4 . The crude mesylated compound and imidazole (2.17 g, 31.86 mmol) were heated at 70°C for 16h. The crude product was purified by silica gel column chromatography (1.5% MeOH in DCM) to give compound **23** as gummy liquid (2.22 g, 33%).

TLC	$R_f = 0.42$ (5% MeOH in DCM, KMnO_4 active).	
^1H NMR	(300 MHz, CDCl_3) δ : 7.41 (s, 1H), 7.29 – 7.20 (m, 5H), 7.03 (s, 1H), 6.83 (s, 1H), 4.45 (s, 2H), 3.86 – 3.77 (m, 1H), 3.41 (t, $J = 6.6$ Hz, 2H), 1.69 – 1.52 (m, 6H), 1.30 – 1.28 (m, 24H), 0.86 – 0.81 (m, 3H).	
^{13}C NMR	(75.46 MHz, CDCl_3) 138.69, 136.43, 129.39, 128.26, 127.5, 127.37, 116.30, 72.78, 70.41, 58.59, 36.28, 31.76, 29.72, 29.38, 29.34, 29.32, 29.29, 29.17, 29.14, 26.12, 26.02, 22.59, 14.07.	
MS (EI)	m/z : 426 $[\text{M}]^+$ (0.9%), 270 (5.0%), 203 (10.4%), 193 (13.7%), 105 (28.4%), 92 (10.5%), 91 (100%), 69 (54.3%).	
HRMS	Calculated: 426.3610	Found: 426.36156

Synthesis of 10-(1*H*-imidazol-1-yl)octadecan-1-ol (**24**)



Compound **23** (1.5 g, 3.52 mmol) and 10% Pd on carbon (200 mg) were taken in EtOH, then it was hydrogenated under hydrogen balloon at reflux for 2 days. The reaction was filtered to remove Pd carbon and filtrate was concentrated. The crude product was purified by silica gel column (3% MeOH in DCM) to give compound **24** (0.96 g, 81%) as gummy liquid.

TLC	$R_f = 0.55$ (10% MeOH in DCM, KMnO_4 and Iodine active).
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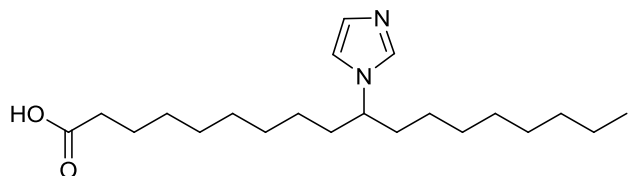
¹H NMR (300 MHz, CDCl₃) δ: 7.43 (s, 1H), 7.03 (s, 1H), 6.86 (s, 1H), 3.90 – 3.80 (m, 1H), 3.59 (t, *J* = 6.9 Hz, 2H), 3.14 (s, 1H), 1.75 – 1.61 (m, 4H), 1.56 – 1.47 (m, 2H), 1.25 – 1.06 (m, 24H), 0.86 – 0.82 (m, 3H).

¹³C NMR (151 MHz, CDCl₃) δ: 136.38, 129.18, 116.42, 62.50, 58.75, 36.28, 36.24, 32.75, 31.75, 29.31, 29.26, 29.18, 29.13, 29.04, 26.03, 25.94, 25.71, 22.58, 14.05.

MS (EI) *m/z* : 336 [M]⁺ (4.6%), 306 (7.9%), 223 (23.3%), 221 (14.1%), 194 (14.9%), 193 (66.8%), 123 (11%), 122 (73.9%), 105 (87.6%), 77 (56.7 %), 69 (100%), 55 (32.9%).

HRMS Calculated: 336.3141 Found: 336.31355

Synthesis of 10-(1*H*-imidazol-1-yl)octadecanoic acid (**25**)

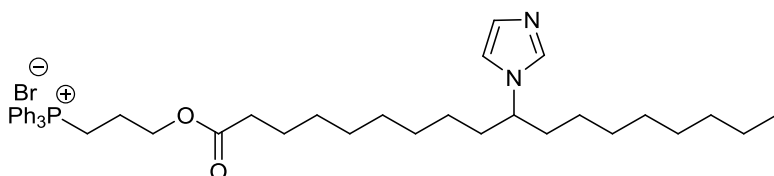


Jones reagent (0.75 ml) was added to a stirred solution of compound **24** (210 mg, 0.63 mmol) in acetone (5 ml) and water (2.5 ml) at 0°C and stirring continued for 3h at room temperature. The reaction mixture was concentrated and diluted with water (10 ml), and extracted with DCM (3 x 10 ml), combined organic layers were washed with brine solution, dried on MgSO₄ and concentrated. The crude compound was purified by silica gel column (3-5% MeOH in DCM) to give the compound **25** (185 mg, 84%) as gummy liquid.

Jones Reagent Preparation: 1 g of CrO₃ was dissolved in con.H₂SO₄ (1 ml), then which was diluted with water (3 ml) at 0°C

TLC	$R_f = 0.5$ (10% MeOH in DCM, KMnO_4 and Iodine active).
^1H NMR	(300 MHz, CDCl_3) δ : 11.23 (s, 1H), 7.69 (s, 1H), 7.12 (s, 1H), 6.88 (s, 1H), 3.94 – 3.85 (m, 1H), 2.34 – 2.29 (m, 2H), 1.78 – 1.58 (m, 6H), 1.26 – 1.07 (m, 22H), 0.89 – 0.74 (m, 3H).
^{13}C NMR	(151 MHz, CDCl_3) δ : 176.76, 136.07, 127.48, 116.65, 59.34, 41.46, 36.20, 36.15, 34.85, 31.78, 29.70, 29.32, 29.29, 29.27, 29.26, 29.17, 29.15, 29.09, 29.06, 29.02, 28.94, 26.03, 25.96, 25.03, 22.61, 14.09.
MS (EI)	m/z : 336 $[\text{M}]^+$ (2.7%), 238 (12.5%), 237 (67.5%), 194 (18.1%), 193 (100%), 95 (19.8%), 69 (90.4 %), 55 (32.6%), 41 (32.7%).

Synthesis of (3-((10-(1*H*-imidazol-1-yl)octadecanoyl)oxy)propyl)triphenylphosphonium bromide (TPP-10-ISA)

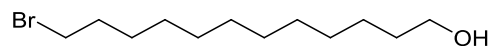


DCC (85 mg, 0.414 mmol) was added to a stirred solution of compound **25** (145 mg, 0.414 mmol), compound **5** (166 mg, 0.414 mmol) and DMAP (5 mg, 0.04 mmol) in dry DCM (5 ml) at 0°C under nitrogen gas. The reaction mixture was stirred at room temperature for 16h. The reaction mixture was filtered to remove the formed DCU and filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column (4-5% MeOH in DCM) to give compound **TPP-10-ISA** (235 mg, 77%) as gummy liquid.

TLC	$R_f = 0.45$ (10% MeOH in DCM, KMnO_4 and iodine active).
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¹ H NMR	(300 MHz, CDCl ₃) δ: 7.89 – 7.66 (m, 15H), 7.54 (s, 1H), 7.07 (s, 1H), 6.92 (s, 1H), 4.36 (t, <i>J</i> = 6.3 Hz, 2H), 4.05 – 3.86 (m, 3H), 2.22 (t, <i>J</i> = 7.5 Hz, 2H), 2.06 – 1.94 (m, 2H), 1.73 – 1.71 (m, 4H), 1.53 – 1.48 (m, 2H), 1.26 – 1.03 (m, 22H), 0.87 – 0.83 (m, 3H).
¹³ C NMR	(75 MHz, CDCl ₃) δ 173.40, 135.15, 135.11, 133.78, 133.64, 130.62, 130.45, 118.62, 117.47, 63.18, 62.95, 58.93, 36.29, 36.24, 34.08, 31.76, 29.32, 29.19, 29.13, 29.04, 29.00, 28.95, 26.04, 25.98, 24.71, 22.58, 22.31, 22.26, 20.11, 19.41, 14.06 .
MS (EI)	m/z: 654 (10.7%), 653 (23.2%), 109 (13.3%), 95 (26.5%), 83 (24.9%), 81 (26.1%), 71 (26.4%), 69 (100%), 57 (68 %), 55 (84%), 43 (76.8%), 41 (68.6%).
HRMS	Calculated: 653.4230 Found: 653.42797

Synthesis of 12-bromododecan-1-ol (**27**)



Dodecane-1, 12-diol **26** (8.365 g, 42.75 mmol) , 48% HBr in water (9.7 ml, 85.50 mmol) and toluene (120 ml) were refluxed for 3 h. The reaction mixture was concentrated, crude compound was dissolved in DCM (150 ml), washed with water, NaHCO₃ brine solution, dried over MgSO₄ and concentrated. The crude product was purified by silica gel column (5% EtOAc in Hexane) to obtain compound **27** as off-white low melting solid (8.87 g, 78%)

TLC R_f = 0.55 (10% EtOAc in Hexane, KMnO₄ active.)

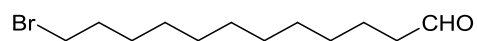
¹H NMR (300 MHz, CDCl₃) δ: 3.63 (t, *J* = 6.6 Hz, 2H), 3.40 (t, *J* = 6.9 Hz, 2H), 3.85 (m, 2H), 1.59 – 1.51 (m, 2H), 1.44 – 1.27 (m, 16H).

¹³C NMR (75 MHz, CDCl₃) δ: 62.91, 34.00, 32.82, 32.74, 29.56, 29.50, 29.41, 29.22, 28.74, 28.15, 25.73.

MS (EI) *m/z* 220 (3.6%), 164 (8.7%), 162 (8.1%), 150 (13.7%), 148 (13.6%), 97 (31.9%), 83 (45.4%), 82 (58.7%), 69 (66.7%), 68 (54.2%), 55 (100%), 41 (57.9%).

HRMS Calculated: 264.1089 Found: [M-18]⁺=246.09741

Synthesis of 12-bromododecanal (**28**)



PCC (7.32 g, 33.93 mmol) was added portion wise to a stirred solution of compound **27** (6.0 g, 22.62 mmol) in dry DCM (90 ml) at 0°C, and then stirring continued at room temperature for another 4h. The reaction mixture was concentrated and purified by silica gel column (6% EtOAc in Hexane) to offere compound **28** as colorless liquid (4.3 g, 72%).

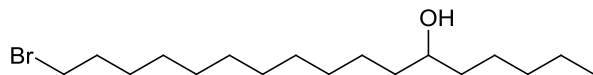
TLC *R_f* = 0.45 (10 EtOAc in Hexane, KMnO₄ active).

¹H NMR (300 MHz, CDCl₃) δ: 9.76 (t, *J* = 2.1 Hz, 1H), 3.41 (t, *J* = 6.9 Hz, 2H), 2.42 (m, 2H), 1.85(m, 2H), 1.63 (m, 2H), 1.44 – 1.28 (m, 14H).

¹³C NMR (75 MHz, CDCl₃) δ: 202.94, 43.88, 34.01, 32.80, 29.40, 29.36, 29.33, 29.30, 29.19, 29.12, 29.03, 28.72, 28.58, 28.14, 24.71, 22.05.

MS (EI) *m/z* [M+1]⁺ 263 (1.5%), 261 (1.6%), 220 (9.7%), 218 (13.7%), 150 (14.5%), 148 (14.6%), 135 (10.5%).

Synthesis of 17-bromoheptadecan-6-ol (**29**)



1-Bromo pentane (2.54 ml, 20.51 mmol) in THF (5 ml) was added dropwise to a stirred suspension of Mg metal (0.54 g, 22.23 mmol) and catalytic amount of iodine in dry THF (15 ml) at 75°C and stirring continued another 30 minutes. The generated Grignard reagent was cooled to room temperature and added slowly to a stirred solution of compound **38** (4.5 g, 17.09 mmol) in THF (40 ml) at 0°C. The reaction was stirred at room temperature for 1.5 hours. The reaction was quenched with NH₄Cl solution, extracted with EtOAc (3x50 ml), combined organic layers were washed with brine solution, dried on MgSO₄ and concentrated. The crude compound was purified over silica gel column chromatography (8% EtOAc in Hexane) to give compound **29** off-white low melting solid (3.34g, 58%).

TLC R_f = 0.54 (10 EtOAc in Hexane, KMnO₄ active).

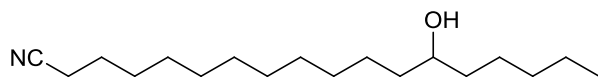
¹H NMR (300 MHz, CDCl₃) δ : 3.60 – 3.56 (m, 1H), 3.41 (t, J = 6.9 Hz, 2H), 1.85 (m, 2H), 1.47 – 1.28 (m, 28H), 0.89 (m, 3H).

¹³C NMR (75 MHz, CDCl₃) δ : 71.98, 37.47, 37.45, 34.02, 32.83, 31.92, 29.70, 29.59, 29.54, 29.42, 28.75, 28.17, 25.65, 25.33, 22.65, 14.05.

MS (EI) m/z : [M+1]⁺ 263 (1.5%), 261 (1.6%), 220 (9.7%), 218 (13.7%), 150 (14.5%), 148 (14.6%), 135 (10.5%).

HRMS Calculated: 264.1089 Found: [M-18]⁺ = 246.09741

Synthesis of 13-hydroxyoctadecanenitrile (**30**)



Compound **29** (1.0 g, 2.98 mmol), NaCN (175 mg, 3.58 mmol) in dry DMF were heated at 90°C for 16 hours. The reaction mixture was cooled to room temperature, and then diluted with diethyl ether (100 ml), organic layer was washed with cold water (3x20 ml), brine solution, dried over MgSO₄ and concentrated. The crude product was purified over silica gel column (10% EtOAc in Hexane) to give compound **30** as colorless liquid (0.77 g, 92%).

TLC R_f = 0.55 (20% EtOAc in Hexane, KMnO₄ and iodine active).

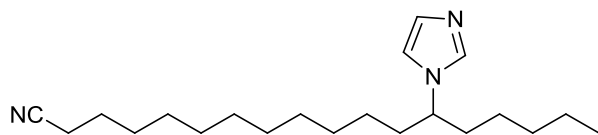
¹H NMR (300 MHz, CDCl₃) δ : 3.60 – 3.56 (m, 1H), 3.41 (m, 2H), 1.85 (m, 2H), 1.47 – 1.28 (m, 27H), 0.92 – 0.87 (m, 3H).

¹³C NMR (75 MHz, CDCl₃) δ : 119.82, 71.88, 62.51, 37.42, 34.83, 31.91, 29.67, 29.55, 29.48, 29.43, 29.25, 28.71, 28.61, 25.62, 25.32, 22.63, 18.88, 17.07, 14.03, 13.84.

MS (EI) m/z [M-18]⁺ 263 (9.8%), 211 (18.6%), 210 (100%), 136 (25.5%), 122 (29.9%), 100 (30.3%), 97(32.4%), 83 (46.2%), 55 (66.5%).

HRMS Calculated: 281.2719 Found: [M-18]⁺ = 263.26080

Synthesis of 13-(1*H*-imidazol-1-yl)octadecanenitrile (**31**)



Methanesulfonyl chloride (1.3 ml, 16.78 mmol) was added to a stirred solution of compound **30** (4.5 g, 15.99 mmol), TEA (2.9 ml, 20.78 mmol) and DMAP (0.19 g, 1.59 mmol) in dry DCM (70 ml) at 0°C under N₂. The reaction mixture was stirred at room temperature for another 2h. The reaction mixture was diluted with DCM (100 ml), organic layer was washed with 1N HCl, water and brine solution, dried over MgSO₄ and concentrated under reduced pressure. This crude compound was mixed with imidazole (2.18 g, 31.97 mmol) and heated at 70°C for 16 hours under N₂. The crude product was purified by silica gel column chromatography (2% MeOH in DCM) to give compound **31** as light yellow liquid (1.64 g, 32 %).

TLC R_f = 0.58 (10% MeOH in DCM, KMnO₄ and iodine active).

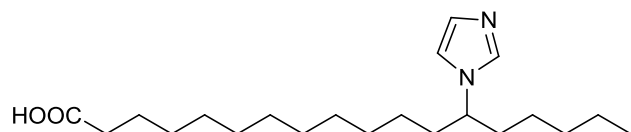
¹H NMR (300 MHz, CDCl₃) δ : 7.44 (s, 1H), 7.05 (s, 1H), 6.87 (s, 1H), 3.86 (m, 1H), 2.31 (t, J = 7.2 Hz, 2H), 1.73 – 1.58 (m, 6H), 1.41 (m, 2H), 1.25 – 1.05 (m, 20H), 0.85 – 0.80 (m, 3H).

¹³C NMR (75 MHz, CDCl₃) δ : 136.49, 129.37, 119.83, 116.39, 58.68, 36.30, 36.27, 31.35, 29.34, 29.19, 29.16, 28.68, 28.59, 26.03, 25.71, 25.31, 22.39, 17.08, 13.91.

MS (EI) m/z [M]⁺ 331 (3.9%), 261 (66.3%), 152 (17.4%), 151 (100%), 69 (50.5%), 55 (20.9%), 41 (23.7%).

HRMS Calculated: 331.2987 Found: [M-18]⁺ = 331.29944

Synthesis of 13-(1*H*-imidazol-1-yl)octadecanoic acid (**32**)



NaOH (332 mg, 8.30 mmol) was added to a stirred solution of compound **31** (550 mg, 1.66 mmol) in EtOH (10 ml) and water (5 ml) and the reaction mixture was refluxed for 16. The reaction mixture was concentrated and acidified with 1N HCl to pH 4, extracted with DCM (4 x 30 ml), washed with brine solution, dried over MgSO₄ and concentrated. The crude product was purified by silica gel column (4% MeOH in DCM) to give compound **32** as thick oil (470 mg, 81%).

TLC R_f = 0.52 (10% MeOH in DCM, KMnO₄ and iodine active).

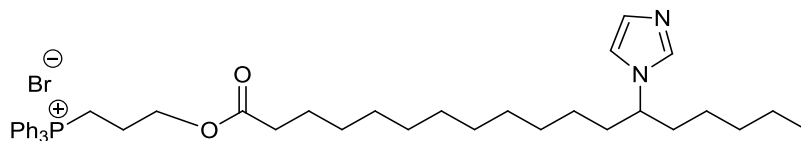
¹H NMR (300 MHz, CDCl₃) δ : 9.81 (s, 1H), 8.60 (s, 1H), 7.34 (s, 1H), 7.05 (s, 1H), 4.20 – 4.10 (m, 1H), 2.31 (t, J = 7.5 Hz, 2H), 1.85 – 1.72 (m, 4H), 1.66 – 1.59 (m, 2H), 1.22 – 1.20 (m, 22H), 1.06 (br, 2H), 0.86 – 0.81 (m, 3H).

¹³C NMR (75 MHz, CDCl₃) δ : 177.81, 60.96, 35.76, 34.44, 31.21, 29.18, 29.13, 29.01, 28.97, 28.90, 25.77, 25.55, 24.88, 22.32, 13.88.

MS (EI) m/z : 351 (2.8%), 350 (8.0%), 230 (14.7%), 222 (20.9%), 221 (100%), 216 (37.8%), 215 (67.7%), 209 (60.4%), 202 (65.4%), 201 (41.1%).

HRMS Calculated: 350.5386 Found: 350.2936.

Synthesis of (3-((13-(1*H*-imidazol-1-yl)octadecanoyl)oxy)propyl)triphenylphosphonium bromide (TPP-13-ISA)



DCC (215 mg, 1.041 mmol) was added to a stirred solution of compound **32** (365 mg, 1.041 mmol) and compound **5** (418 mg, 1.041 mmol) and DMAP (catalytic amount) in DCM (10 ml) at 0°C under nitrogen gas and stirring continued overnight at room temperature. The reaction mixture was filtered and concentrated. The crude compound was purified by silica gel column (5% MeOH in DCM) to give compound **TPP-13-ISA** (600 mg, 78%) as gummy liquid.

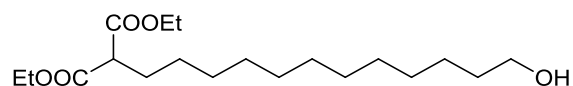
¹H NMR (600 MHz, CDCl₃) δ: 7.89 – 7.84 (m, 6H), 7.82 – 7.79 (m, 3H), 7.72 – 7.69 (m, 6H), 7.48 (s, 1H), 7.06 (s, 1H), 6.90 (s, 1H), 4.38 – 4.36 (m, 2H), 4.02 – 3.97 (m, 3H), 2.42 (t, J = 7.8 Hz, 4H), 2.02 – 2.00 (m, 2H), 1.73 – 1.68 (m, 4H), 1.54 – 1.52 (m, 2H), 1.25 – 1.06 (m, 22H), 0.85 – 0.82 (m, 3H).

¹³C NMR (151 MHz, CDCl₃) δ: 173.49, 135.16, 133.76, 133.70, 133.60, 130.52, 118.33, 117.76, 68.12, 63.00, 58.83, 36.31, 36.28, 34.16, 31.37, 29.47, 29.43, 29.37, 29.23, 29.19, 29.09, 26.07, 25.74, 24.83, 22.43, 22.28, 19.96, 19.61, 13.96.

MS (EI) m/z: [M+1]⁺ (16.2%), [M]⁺653 (34.5%), 183 (11.5%), 95 (15.1%), 77 (10.1%), 69 (100.0%), 57 (18.5%), 55 (31.2%), 43 (24.2%), 41 (26.4%).

HRMS Calculated: 653.4230 Found: 653.42199

Synthesis of diethyl 2-(12-hydroxydodecyl)malonate (**33**)

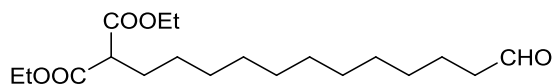


Diethylmalonate (7.52 ml, 48.316 mmol) was added to a stirred solution of compound **27** (6.54 g, 24.658 mmol), K₂CO₃ (8.52 g, 61.645 mmol), KI (0.409 g, 2.466 mmol) in dry acetonitrile (100 ml) and heated at 90°C for 48h. The reaction mixture was filtered to remove the solid waste

and filtrate was concentrated. The crude compound was purified by silica gel column (7-10 % EtOAc in hexane) to give the compound **33** (5.35 g, 63%) as colorless liquid.

TLC	$R_f = 0.43$ (30% EtOAc in Hexane, Iodine and KMnO_4 active).
^1H NMR	(300 MHz, CDCl_3) δ : 4.20-4.13 (q, $J = 7.2$ Hz, 4H), 3.60 (t, $J = 6.6$ Hz, 2H), 3.29 (t, $J = 7.5$ Hz, 1H), 1.87 – 1.82 (m, 2H), 1.74 (s, 1H), 1.56 – 1.49 (m, 2H), 1.28 – 1.22 (m, 24H).
^{13}C NMR	(75 MHz, CDCl_3) δ : 169.59, 62.92, 61.20, 52.04, 32.75, 29.54, 29.49, 29.43, 29.39, 29.24, 29.15, 28.69, 27.26, 25.71, 14.03.
MS (EI)	m/z : 344 (4.7%), 173 (56.8%), 160 (100%), 55 (40.5%), 45 (47.1%), 44 (46.6%).
HRMS	Calculated: 344.2563 Found: 344.25495

Synthesis of diethyl 2-(12-oxododecyl)malonate (**34**)



PCC (3.67g, 17.025mmol) was added portion-wise to a stirred solution of compound **33** (3.91g, 11.35 mmol) in dry DCM (60 ml) at 0°C. The reaction was allowed to room temperature and stirring continued for another 3h. The reaction mixture was concentrated under reduced pressure and purified by the silica gel column (8% EtOAc in Hexane) to give the compound **34** (2.84 g, 73%) as colorless liquid.

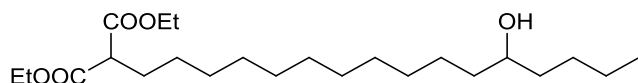
TLC	$R_f = 0.4$ (20% EtOAc in Hexane, iodine and KMnO_4 active).
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¹H NMR (300 MHz, CDCl₃) δ: 9.73 (t, *J* = 1.8 Hz, 3H), 4.20 – 4.13 (q, *J* = 7.2 Hz, 4H), 3.28 (t, *J* = 7.8 Hz, H), 2.42 – 2.36 (m, 2H), 1.89 -1.82 (m, 2H), 1.64 – 1.55 (m, 2H), 1.28 – 1.26 (m, 22H)

¹³C NMR (75 MHz, CDCl₃) δ: 202.81, 169.53, 61.16, 52.01, 43.84, 29.42, 29.39, 29.32, 29.27, 29.22, 29.13, 29.09, 28.68, 27.24, 22.02, 14.02.

MS (FAB) m/z: 299 (5.9%), 173 (60.9%), 133 (15.7%), 127 (10.3%), 98 (20.7%), 95 (18.9%), 69 (23.2%), 55 (62.2%), 43 (34.2%), 41 (39.4%).

Synthesis of diethyl 2-(12-hydroxyhexadecyl)malonate (**35**)



1-Bromobutane (0.92ml, 8.512mmol) in dry THF (5ml) was added slowly to a stirred suspension of Mg metal (0.25g, 10.059mmol) and catalytic amount of iodine in dry THF (10 ml) at reflux temperature, stirred for 30 min after completion of addition. The prepared Grignard reagent was added drop wise to a stirred solution of compound **34** (2.65 g, 7.738 mmol) in THF (30 ml) at -10 to -15°C and stirred at this temperature for 30 minutes. The reaction mixture was slowly warmed to room temperature and stirring continued for another one hour. The reaction was quenched with saturated ammonium chloride solution (10 ml), diluted with water (20 ml), extracted with diethyl ether (3 x 50 ml), dried on anhydrous MgSO₄ and concentrated. The crude product is purified by silica gel chromatography (4 – 6% EtOAc in hexane) to offer compound **35** (1.94g, 62.5%) as colorless liquid.

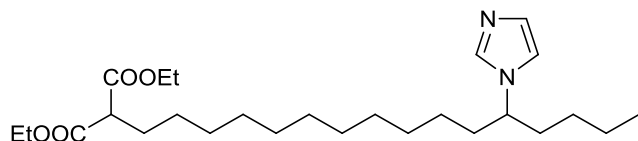
TLC R_f = 0.53 (20% EtOAc in Hexane, iodine and KMnO₄ active).

¹H NMR (300 MHz, CDCl₃) δ: 4.21-4.13 (m, 4H), 3.58 – 3.54 (m, 1H), 3.29 (t, *J* = 7.5 Hz, 1H), 1.88 – 1.83 (m, 2H), 1.74 (s, 1H), 1.51 – 1.22 (m, 33H), 0.89 (t, *J* = 6.9 Hz, 3H).

¹³C NMR (75 MHz, CDCl₃) δ: 169.57, 71.90, 61.19, 52.04, 37.46, 37.15, 29.68, 29.58, 29.53, 29.45, 29.26, 29.17, 28.70, 27.82, 27.28, 25.62, 22.74, 14.05.

MS (EI) m/z: [M – 18]⁺ 382 (1.5%), 343 (26.8%), 297 (14.9%), 251 (29.7%), 173 (100.0%), 161 (15.0%), 160 (77.8%), 98 (23.0%), 87 (20.5%), 73 (19.0%), 69 (47.6%), 55 (51.3%), 43 (26.6%), 41 (35.3%).

Synthesis of diethyl 2-(12-(1*H*-imidazol-1-yl)hexadecyl)malonate (**36**)

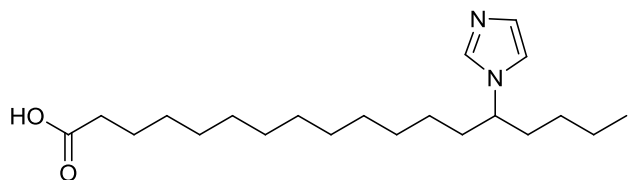


Methanesulfonyl chloride (0.36 ml, 4.587 mmol) was added slowly to a stirred solution of compound **35** (1.75 g, 4.369 mmol), TEA (0.79 ml, 5.679 mmol) and DMAP (53 mg, 0.437 mmol) in dry DCM (20 ml) at 0°C. The reaction mixture was stirred at room temperature for 2h. The reaction mixture was diluted with DCM (75 ml), washed with water (50 ml), 0.5 N HCl (10 ml) and brine solution (20 ml), dried on dry MgSO₄ and concentrated. The crude compound was mixed with imidazole (595 mg, 8.737 mmol) and stirred at 75°C for 16h. The crude compound was purified by silica gel column chromatography (2% MeOH in DCM) to give the compound **36** (640mg, 32.5%) as light brown color liquid.

TLC R_f = 0.63 (5% MeOH in DCM, Iodine and KMnO₄ active).

^1H NMR	(300 MHz, CDCl_3) δ : 7.44 (s, 1H), 7.04 (s, 1H), 6.86 (s, 1H), 4.19 – 4.12 (q, J = 7.2 Hz, 4H), 3.90 – 3.80 (m, 1H), 3.27 (t, J = 7.5 Hz, 1H), 1.85 – 1.83 (m, 2H), 1.73 – 1.64 (m, 2H), 1.26 – 1.00 (m, 29H), 0.83 – 0.78(m, 3H).
^{13}C NMR	(75 MHz, CDCl_3) δ : 169.54, 136.40, 129.21, 116.41, 61.17, 58.70, 52.01, 36.27, 35.98, 29.40, 29.32, 29.22, 29.14, 28.68, 28.17, 27.25, 26.02, 22.24, 14.03, 13.82.
MS (EI)	m/z : $[\text{M} + 1]^+$ (3.2%), $[\text{M}]^+$ (8.7%), 405 (17.8%), 393 (26.2%), 291 (40.6%), 137 (55.6%), 95 (23.9%), 81 (22.4%), 69 (100%), 68 (42.5%), 55 (44.3%), 41 (40.9%).
HRMS	Calculated: 450.3458 Found: 450.3474

Synthesis of 14-(1*H*-imidazol-1-yl)octadecanoic acid (**37**)



NaOH (147 mg, 3.684 mmol) was added to a stirred solution of compound **36** (415 mg, 0.921 mmol) in a 3:1 mixture of methanol (9 ml) and water (3 ml) at 0°C and stirred at room temperature for 3h. The reaction mixture was concentrated under reduced pressure to remove MeOH and adjust the pH of mixture to 5 with 1N HCl and extracted with DCM (3 x 25 ml), dried on anhydrous MgSO_4 and concentrated under reduced pressure. The crude compound was obtained as light yellow color liquid. This crude compound was heated at 170°C to remove the one carboxyl acid group from di-carboxylic acid by the release of CO_2 . The crude product was

purified by silica gel column chromatography (3 to 5% of MeOH in DCM) to give the compound **37** (221 mg, 68%) as pale yellow gummy liquid.

TLC R_f = 0.48 (10% MeOH in DCM, iodine and KMnO_4 active).

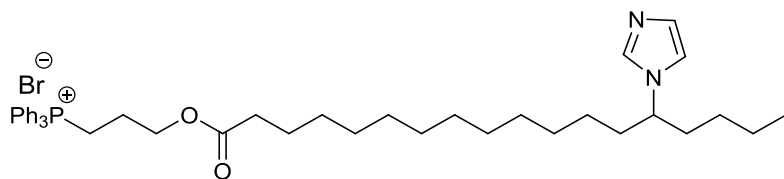
^1H NMR (300 MHz, CDCl_3) δ : 10.28 (s, 1H), 7.70 (s, 1H), 7.12 (s, 1H), 6.89 (s, 1H), 3.95 – 3.85 (m, 1H), 2.33 (t, J = 7.5 Hz, 2H), 1.80 – 1.60 (m, 6H), 1.37 – 1.00 (m, 23H), 0.85 (t, J = 7.2 Hz, 3H).

^{13}C NMR (151 MHz, CDCl_3) δ : 177.89, 136.12, 127.48, 116.71, 59.36, 36.16, 35.92, 34.83, 29.40, 29.32, 29.29, 29.26, 29.20, 29.15, 29.06, 29.06, 28.18, 25.96, 25.07, 22.30, 22.27, 13.86.

MS (EI) m/z : $[\text{M}]^+$ 350 (1.9%), 293 (34.9%), 151 (11.4%), 138 (16.9%), 137 (55.6%), 95 (15.6%), 69 (100%), 55 (31.5%), 43 (22.9%), 41 (30%).

HRMS Calculated: 350.2933 Found: 350.29349

Synthesis of (3-((14-(1*H*-imidazol-1-yl)octadecanoyl)oxy)propyl)triphenylphosphonium bromide (TPP-14-ISA)



DCC (104 mg, 0.502 mmol) was added to a stirred solution of compound **37** (176 mg, 0.502 mmol), compound **5** (201 mg, 0.502 mmol) and DMAP (6 mg, 0.05 mmol) in dry DCM (10 ml) at 0°C and stirred for 16 at room temperature. The reaction mixture was filtered to remove the formed DCU and concentrated. The crude product was purified by silica gel column

chromatography (4% MeOH in DCM) to give the compound **TPP-14-ISA** (263 mg, 71%) as colorless gummy liquid.

TLC R_f = 0.35 (10% MeOH in DCM, iodine and KMnO_4 active).

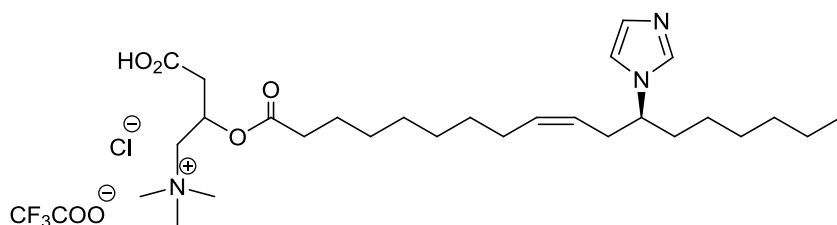
^1H NMR (300 MHz, CDCl_3) δ : 7.89 – 7.66 (m, 15H), 7.49 (s, 1H), 7.06 (s, 1H), 6.90 (s, 1H), 4.36 (t, J = 6.3 Hz, 2H), 4.05 – 3.89 (m, 3H), 2.23 (t, J = 7.5 Hz, 2H), 2.05 – 1.96 (m, 2H), 1.76 – 1.66 (m, 4H), 1.55 – 1.50 (m, 2H), 1.31 – 1.03 (m, 22H), 0.83 (t, J = 7.5 Hz, 3H).

^{13}C NMR (75 MHz, CDCl_3) δ : 173.44, 135.13, 135.10, 133.79, 133.66, 130.61, 130.44, 118.65, 117.51, 63.17, 62.93, 58.87, 36.29, 35.99, 34.15, 29.49, 29.43, 29.37, 29.22, 29.19, 29.09, 28.19, 26.04, 24.82, 22.27, 20.13, 19.43, 13.85.

MS (FAB) m/z : $[\text{M}+1]^+$ 654 (11.7%), 653 (24.1%), 183 (12.1 %), 95 (16.5 %), 77 (16.5 %), 69 (100%), 57 (33.2%), 43 (46%), 41 (51%).

HRMS Calculated: 653.4230 Found: 653.42143

Synthesis of mono(2-(((*S,Z*)-12-(1*H*-imidazol-1-yl)octadec-9-enoyl)oxy)-3-carboxy-*N,N,N*-trimethylpropan-1-aminium) mono(2,2,2-trifluoroacetate) monochloride (CSE-12-IOA)



Compound **3** (0.3g, 0.86 mmol) was dissolved in 5 ml of dry DCM, then one drop of dry DMF and oxalyl chloride (0.11ml, 1.29 mmol) were added under nitrogen atmosphere, stirred for 1h at

room temperature. The reaction mixture was concentrated, co-evaporated with dry chloroform (2 x 10 ml), dried under vacuum for 1h. Carnitine hydrochloride **39** (0.153g, 0.77 mmol) in 1 ml of TFA was added slowly to the previously prepared acid chloride **40** and heated at 45°C for 16 h. It was cooled in ice bath, triturated with 10ml of acetone. After 2 hours the precipitated material was removed by centrifugation. Anhydrous ether was added drop wise to the supernatant until a slight cloudiness appeared, then gummy liquid was formed instead of crystals, then this was centrifuged. The gummy material was washed with 3:1 ratio of diethyl ether and acetonitrile to give the compound **CSE-12-IOA** (405 mg, 81%) as dark brown gummy material.

TLC R_f = 0.25 (10% water in MeOH, KMnO₄ active).

¹H NMR (600 MHz, DMSO – d₆) δ : 9.25 (s, 1H), 7.93 (s, 1H), 7.20 (s, 1H), 5.47 – 5.37 (m, 2H), 5.21 – 5.17 (m, 1H), 4.42 – 4.38 (m, 1H), 3.83 – 3.79 (m, 1H), 3.65 – 3.64 (m, 1H), 3.13 (s, 9H), 2.69 – 2.68 (m, 2H), 2.59 – 2.53 (m, 1H), 2.34 – 2.29 (m, 2H), 1.89 – 1.83 (m, 4H), 1.52 – 1.50 (m, 2H), 1.27 – 1.12 (m, 16H), 0.84 – 0.82 (m, 2H).

¹³C NMR (151 MHz, CDCl₃) δ : 172.60, 171.00, 158.83, 158.62, 158.41, 120.95, 120.47, 118.42, 116.44, 67.54, 65.37, 61.00, 53.44, 37.59, 34.76, 34.06, 31.43, 31.16, 29.34, 29.30, 29.24, 29.18, 28.86, 28.83, 28.45, 25.61, 25.54, 24.57, 22.39, 14.34.

MS (FAB+) m/z: [M⁺] 492 (37.7 %), 363 (19.1 %), 349 (15 %), 149 (22.6 %), 144 (24.2 %), 100 (24%), 95 (25.7 %), 69 (100 %).

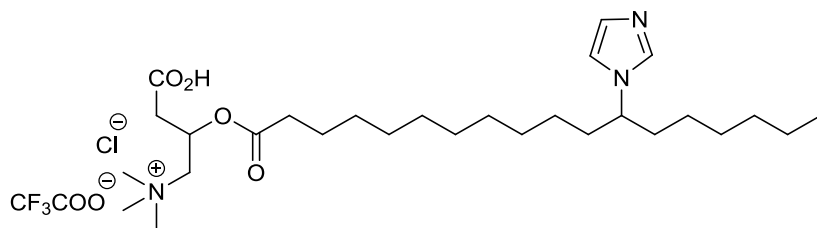
HRMS Calculated: 492.3952 Found: 492.35240

CHN analysis: Actual value: C: 54.94 %; H: 7.41%; N: 6.86%.

Experimental value: C: 54.32, H: 7.89%; N: 6.57%.

Elemental analysis result has confirmed that CSE-12-IOA is present approximately, as a salt of HCl and TFA in 1: 1 ratio.

Synthesis of mono(2-((12-(1*H*-imidazol-1-yl)octadecanoyl)oxy)-3-carboxy-*N,N,N*-trimethylpropan-1-aminium) mono(2,2,2-trifluoroacetate) monochloride (CSE-12-ISA)



To a stirred solution of compound **9** (0.45 g, 1.28 mmol) in 10 ml of dry DCM, cooled to 0°C, then one drop of dry DMF and oxalyl chloride (0.165 ml, 1.93 mmol) were added under nitrogen atmosphere and stirred for 1h at room temperature. The reaction mixture was concentrated, co-distilled with dry chloroform (2x15 ml), dried under vacuum for 1h. Carnitine hydrochloride (0.228 g, 1.16 mmol) in TFA (2 ml) was added to the acid chloride **40** and heated at 45°C for 16h. The reaction was cooled in ice bath, triturated with 15 ml of acetone. After 2 hours the precipitated material was removed by centrifugation. Anhydrous ether was added drop wise to the supernatant until a slight cloudiness appeared, then gummy liquid was formed instead of crystals, then this was centrifuged. The gummy material was washed with 3:1 ratio of diethyl ether and acetonitrile to give the compound **CSE-12-ISA** (620 mg, 83%) as dark brown gummy material.

TLC R_f = 0.25 (10% water in MeOH, KMnO₄ active).

¹H NMR (600 MHz, DMSO - d₆) δ : 9.27 (s, 1H), 7.91 (s, 1H), 7.75 (s, 1H), 5.49 – 5.43 (m, 1H), 4.36 – 4.33 (m, 1H), 3.83 – 3.79 (m, 1H), 3.65 – 3.63 (m, 2H), 3.10 (s, 9H),

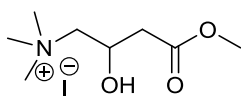
2.69 – 2.68 (m, 2H), 2.34 – 2.27 (m, 2H), 2.08 (s, 2H), 1.80 – 1.78 (m, 4H), 1.52 – 1.49 (m, 2H), 1.25 – 1.11 (m, 21H), 0.94 – 0.93 (m, 2H), 0.84 – 0.82 (m, 3H).

¹³C NMR (151 MHz, DMSO – d₆) δ: 172.60, 171.00, 158.56, 158.53, 135.14, 133.81, 124.36, 120.69, 120.63, 67.54, 65.38, 60.84, 53.44, 37.60, 34.05, 33.17, 31.42, 29.38, 29.07, 28.95, 28.82, 28.46, 27.06, 25.57, 24.57, 22.40, 14.35.

MS (FAB+) m/z: [M+1] 495 (14.1 %), 494 (44.2 %), 144 (33.6 %), 100 (23.9 %), 85 (25.6 %), 69 (100 %), 58 (37.4 %).

HRMS Calculated: 494.3952 Found: 494.36024

Synthesis of 2-hydroxy-4-methoxy-*N,N,N*-trimethyl-4-oxobutan-1-aminium iodide (**41**)



To a stirred suspension of carnitine hydrochloride **39** (2 g, 10.12 mmol) in MeOH (30 ml), 57% HI in water (3 ml) was added to it and refluxed for 6h. The reaction mixture was concentrated. The crude compound was dissolved in acetone (20 ml) then added diethyl ether (60 ml) and required compound precipitated filtered and dried under vacuum. Compound **41** obtained as light yellow color solid (1.7 g, 55 %).

TLC R_f=0.56 (20% MeOH in DCM, Iodine, KMnO₄ and ninhydrine active.)

¹H NMR (300 MHz, DMSO-d₆) δ: 5.64 – 5.62 (d, J = 6.0 Hz, 1H), 4.46 – 4.39 (m, 1H), 3.42 – 3.32 (m, 2H), 3.15(s, 9H), 2.65 – 2.41 (m, 2H).

¹³C NMR (75 MHz, DMSO-d₆) δ: 170.87, 71.78, 69.58, 62.83, 53.90, 52.11.

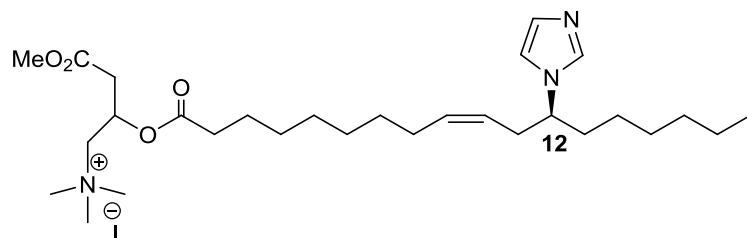
MS (EI) m/z: [M+1]⁺ 177 (9.6%), [M]⁺ 176 (100%), 59 (5.5%), 58 (11.4%).

HRMS

Calculated:176.1281

Found: 176.12737

Synthesis of 2-(((*S,Z*)-12-(1*H*-imidazol-1-yl)octadec-9-enoyl)oxy)-4-methoxy-*N,N,N*-trimethyl-4-oxobutan-1-aminium iodide (CDE-12-IOA)



DCC (89 mg, 0.43 mmol) was added to a stirred solution of compound **3** (150 mg, 0.43 mmol) compound **41** (130 mg, 0.43 mmol), and DMAP (3 mg) in DCM (10 ml) at 0°C. The reaction was stirred at RT for 16h, filtered off the formed DCU and concentrated. The crude product was purified by basic alumina column (3-4% MeOH in DCM) to give the compound **51** (187 mg, 69%) as very thick gummy liquid.

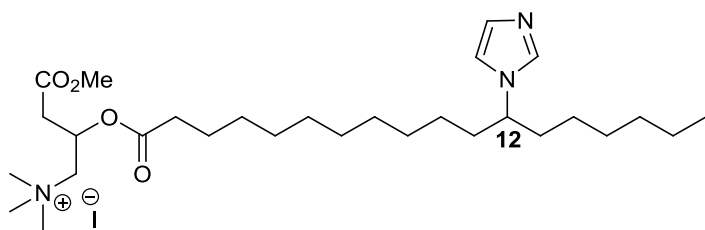
TLC R_f =0.35 (15% MeOH in DCM, iodine, KMnO_4 and ninhydrin active.)

^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 7.59 (s, 1H), 7.19 (s, 1H), 6.85 (s, 1H), 5.52 – 5.46 (m, 1H), 5.38 – 5.30 (m, 1H), 5.20 – 5.12 (m, 1H), 4.21 – 4.00 (m, 1H), 3.85 – 3.71 (m, 2H), 3.67 (s, 3H), 3.09 (s, 9H), 2.79 – 2.77 (m, 2H), 2.51 – 2.49 (m, 2H), 2.46 – 2.38 (m, 2H), 2.35 – 2.29 (m, 2H), 1.88 (s, 2H), 1.76 – 1.68 (m, 2H), 1.53 – 1.48 (m, 2H), 1.27 – 1.17 (m, 15H), 0.97 – 0.91 (m, 1H), 0.80 (m, 3H).

^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 172.55, 169.88, 137.08, 132.60, 128.80, 125.61, 117.56, 67.35, 65.16, 57.76, 53.53, 52.29, 37.35, 35.18, 34.02, 31.54, 29.32, 29.02, 28.93, 28.77, 28.62, 27.10, 25.86, 24.53, 22.40, 14.34.

MS (EI)	m/z: [M+1] ⁺ 507 (22.6%), [M] ⁺ 506 (68.2%), 158 (34.1%), 99 (100%), 95 (25.1%), 83 (24.9%), 69 (100%), 59 (35.4%), 58 (53%), 55 (46.4%), 41 (33.5%).	
HRMS	Calculated: 506.3952	Found: 506.38453

Synthesis of 2-((12-(1*H*-imidazol-1-yl)octadecanoyl)oxy)-4-methoxy-*N,N,N*-trimethyl-4-oxobutan-1-aminium iodide (CDE-12-ISA)



DCC (118 mg, 0.57 mmol) was added to a stirred solution of compound **8** (200 mg, 0.57 mmol) compound **41** (173 mg, 0.57 mmol) and DMAP (4 mg) in dry DCM (10 ml) and stirring continued at room temperature for 16. The reaction mixture was filtered to remove formed DCU and concentrated. The crude compound was purified by basic alumina column (3-4% MeOH in DCM) to offered compound **CDE-12-ISA** (236 mg, 65%) as thick gummy liquid.

TLC	R _f =0.32 (15% MeOH in DCM, KMnO ₄ , iodine and ninhydrin active.)	
¹ H NMR	(300 MHz, DMSO-d ₆) δ 7.61 (s, 1H), 7.16 (s, 1H), 6.88 (s, 1H), 5.51 (m, 1H), 4.04 – 3.94 (s, 1H), 3.84 – 3.73 (m, 2H), 3.62 (s, 3H), 3.13 – 3.09 (s, 9H), 2.79 – 2.76 (m, 2H), 2.34 – 2.28 (m, 2H), 1.70 – 1.63 (m, 4H), 1.52 – 1.48 (m, 2H), 1.2 – 1.17 (m, 21H), 0.94 – 0.92 (m, 2H), 0.82 (m, 3H).	

¹³ C NMR	(75 MHz, DMSO-d ₆) δ: 172.55, 169.88, 137.20, 128.94, 117.35, 67.36, 65.15, 57.71, 53.53, 52.27, 37.34, 35.87, 34.01, 31.56, 29.32, 29.26, 29.10, 28.96, 28.79, 28.63, 25.90.	
MS (EI)	m/z: [M+1] ⁺ 509 (14.5%), [M] ⁺ 508 (44.1%), 158 (22.6%), 99 (48.0%), 83 (18.7%), 69 (100%), 59 (22.6%), 58 (31.7%), 55 (27.2%), 41 (21.7%).	
HRMS	Calculated: 508.3952	Found: 508.40062

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